ANALYSIS OF PROGRAM ACTIVITIES
NATIONAL INSTITUTES OF HEALTH
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NATIONAL INSTITUTE OF
ARTHRITIS AND METABOLIC DISEASES

CLINICAL CENTER

NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

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Analysis of NIH Program Activities

January-December 1955

U.S National Institute of Arthritis and Metabolic Diseases

Laboratory Research

NIAMD laboratory research programs have moved forward steadily on a broad front. Significant accomplishments can be reported not only from a variety of basic studies but also from studies in human disease and metabolism. The highlights of research progress to follow are organized on the same general pattern as in the 1954 report for convenience in following progress in specific areas.

Amino Acids - The multitudinous ways in which amino acids are involved in metabolism and in metabolic diseases are only beginning to be unraveled. As reported previously, animals with experimental diabetes have been found to be unable to convert the amino acid tryptophan to the vitamin nicotinic acid. These studies have been extended to demonstrate that the central defect appears to be inability to remove the alanine side chain residue from certain tryptophan metabolites. An enzyme involved in the transformation of tryptophan has been found to be increased in these diabetic animals. Insulin will normalize the enzyme pattern and abnormal tryptophan metabolism, whereas adrenal steroids (cortisone) have the opposite action. In other studies, the value of synthetic amino acids in human diets has been explored. Using human volunteers on low protein (rice) diets, it has been shown that two of the essential amino acids, lysine and threonine, or a mixture of the 8 amino acids which are indispensable in human diets, or a mixture of certain non-essential amino acids are capable of improving the protein value of this diet. In certain patients however the essential amino acid supplements produced a negative, unfavorable effect. This entirely expected and perhaps significant finding remains to be explained. Because of the important role of 5-hydroxy-tryptophan and its catabolites in central nervous system function, the extremely labile 5,6-dihydroxyindole derivatives have been synthesized. The possible formation of tricyclic derivatives of β - substituted pseudo-tryptophans and -tryptamines to yield 2-carboxy-eseroline derivatives has been demonstrated. A method to synthesize 5-hydroxypipecolic acid from γ-hydroxylysine has been developed. 5-Hydroxypipecolic acid also has been isolated in pure form from dates. The stereochemistry of hydroxyproline, a major constituent of collagen, has been elucidated. The long sought-for lactone of allohydroxyproline as well as other derivatives were synthesized in these studies for testing in collagen forming systems. A new synthesis of formiminoglutamic acid (important in the metabolism of histidine and the B vitamin folic acid) and of formimino-aspartic acid (an intermediate in the metabolism of histamine) has been developed. The pathway of histidine biosynthesis has been almost completely elucidated. The B vitamin (pyridoxal phosphate) is required for one of the steps. Two

completely new compounds, β -aspartyl phosphate and aspartic-β -semialdehyde have been identified as intermediates in the biological transformation of aspartic acid to homoserine. The reality of peptide bond interactions through space has been demonstrated.

Amines - These substances are known to be formed in the human body and certain of them have long been suspected to be involved in "intestinal auto-intoxication". This theory has never been proved but NIAMD scientists have shown that two amines, spermine and spermidine are potent kidney damaging agents. A single injection of these compounds into the renal artery of rabbits leads to progressive degeneration and almost complete atrophy of the kidney in a few months, with, in many instances, elevated blood pressure and focal myocardial necrosis. Spermine, in the presence of a certain enzyme (amine oxidase), is also highly lethal to spermatozoa and certain bacteria. SH-compounds antagonize this action. Chemical methods have been developed to synthesize spermine, and C^{14} and N^{15} labelled putrescine. These compounds have been used to demonstrate the biosynthesis of spermine and spermidine from putrescine. Spermine has been found to be widely distributed in animal and plant cells. Evidence has also been obtained that putrescine is a normal constituent of animal cells (previously thought to be only a product of protein decomposition).

Proteins - These substances are not only essential to life as components in diets but are major structural elements in the body, function as hormones, enzymes, antibodies and in working elements such as muscle. A new protein containing free SH-groups, to be called "sulfhydrotropomyosin", has been discovered and isolated from rabbit muscle. Bovine fibrinogen was prepared in crystalline form and used as test material for the Laki-Lorand factor which has been found to be absent in certain disorders of bone marrow. The muscle enzyme, myokinase, has been obtained in a high state of purity. The amino acid composition of the contractile muscle proteins from a number of sources in different species has been determined. The amino acid composition showed increasing randomness going from highly developed animals to lower ones. Parallel changes in the total electrical charge of the molecule was found. However, the contractile protein myosin obtained from pregnant and non-pregnant human uteri was found to behave similarly to myosin from skeletal muscle. Myosin from rabbits was found to have a high molecular weight ribonucleic acid associated with it. The principal intracellular cation, potassium, was found to be bound to carboxylic acid groups of myosin. The structure and properties of insulin were explored with the guanidination reaction, previously developed for serum albumin and fibrinogen. Varying amounts of SH-groups have been found in serum albumin from different sources and of different ages. The possibility that a reversible thiazoline ring formation might account for these findings is being explored.

Carbohydrates - These substances occupy a key position in the research program of NIAMD since they are a major metabolic fuel as well as components of structural elements in tissues. The broad and systematic studies being conducted found direct application in human

disease during the year. Patients with the congenital disease, galactose diabetes (galactosemia) were found not to possess an enzyme which is essential for the metabolism of galactose (a component of milk sugar). This discovery was made possible by fundamental studies on carbohydrate metabolism conducted by NIAMD scientists. These findings are significant not only because they explain a hitherto obscure metabolic disease, provide a specific diagnostic test which is not dangerous to the patient, but also open the door to further studies as to whether unrecognized inability to metabolize galactose may be a factor in certain presently obscure disease states such as liver cirrhosis, blindness due to cataract and mental retardation. Discoveries in the fundamental studies have been many during the year. Studies on the pentose pathway of carbohydrate metabolism have been extended to the point where the pathway of carbohydrate synthesis from carbon dioxide in photosynthesis has been completely clarified. This is a particularly significant accomplishment since 10 distinct and complex steps are involved in these reactions. From this work it is possible to predict that the maximum efficiency of photosynthesis is 6 quanta per mole of carbon dioxide - long a highly controversial issue. New methods for the preparation of ribulose diphosphate and xylulose 5-phosphate have been developed. The latter compound, first discovered by an NIAMD scientist, has been shown to be involved in the transketolase reaction, indicating an important role in nucleic acid formation and carbon dioxide synthesis. Another newly discovered sugar, 3-ketopentose phosphate has been identified in biological systems. The relative importance of various pathways of carbohydrate metabolism in various species, various tissues, in normal and diabetic animals has been studied further. Brain tissue appears to utilize only one pathway, the Embden-Meyerhof sequence. Liver from diabetic rats metabolizes more via the oxidative (pentose) pathway than do normal animals. The biologically important conversion of fructose-6-phosphate into glucose-6-phosphate has been studied utilizing tracer deuterium. Evidence has been obtained for an opening of the hemiacetal ring, followed by activation of the alpha hydrogen as a proton and subsequent enediol formation as being involved in this reaction. Patients with diabetic acidosis who show an increased insulin requirement have been found to have an insulin antagonist in their serum. The adrenal cortex or lowered serum pH are not involved in this phenomenon. The factor seems to be associated with the α and β serum globulins. The metabolic fate of certain substances closely related to carbohydrates, namely glucuronolactone and glucuronate were studied in man. The principal immediate fate of glucuronolactone is hydrolysis to glucuronate. Only liver seems to have the capacity for this conversion. Neither compound was incorporated into urinary glucuronide. An intermediate in carbohydrate metabolism, 3-phosphoglyceric acid, was found to be converted enzymatically into the previously unknown methylthio-ester, and again enzymatically into the also newly discovered, glyceryl methylthiol ester. These reactions involving CH_2-SH groups constitute a new area in metabolism and may have much significance. The studies on glycogen (the principal animal sugar) metabolism have been extended to muscle as well as liver glycogen. These studies reveal a pattern of metabolic inhomogeneity which is quite unique. Muscle and liver glycogen seem to behave quite differently in that in liver the smaller glycogen molecules are

metabolically more reactive, while in muscle the opposite is true: the peripheral replacement of glucose residues is more rapid on the larger molecules than on smaller ones. Systematic studies in carbohydrate chemistry are being continued. Specially significant accomplishments were the preparation of the crystalline α-hexaacetate of D-altro-heptulose (sedoheptulose); proof of the structure of the second sedoheptulosan as 2,7-anhydro-β-D-altro-heptulofuranose; isolation of what is presumably di-α-D-gluco-heptulopyranose 2,1':2',1-dianhydride; and the preparation and alkaline degradation of phenyl α-D-gluco-heptulopyranoside to 2,7-anhydro-β-D-gluco-heptulopyranose. Studies in the ribose series have led to the identification of 2,3-O-benzylidene-D-ribofuranose which may prove useful in the synthesis of other desired ribose derivatives. Studies of certain highly reactive sugar esters have produced evidence that substances assigned 1,2-O-(1-hydroxyalkylidene) structures are, in fact, 1-acyl sugars having the hydroxyl group at C_2 free. A type of reaction not hitherto observed was found when the benzyl group of 2,3-(1-benzyloxybenzylidene)-β-D-fructofuranose tribenzoate was readily replaced by an ethyl group through the action of weakly acidic ethanol. The interesting and unique structural features of the carbohydrate moieties of stevioside have led to the surprising discovery of migration of a sterically hindered acyl group from the α C_1 to the C_2 position in glucopyranose indicating that the normal acyl groups at the α C_1 position must be exceedingly labile, if indeed they exist at all. An analogous discovery was made almost simultaneously by an outside researcher in which migration of the phosphate group in the D-ribofuranose series was observed.

Vitamins - Studies related to these vital substances continue to receive attention not only because of their essentiality as dietary components but also because they function as catalysts in a wide spectrum of biochemical reactions. Picolinic acid (closely related chemically to the vitamin nicotinic acid) has been found, for the first time, to be formed in biological systems and evidence has been obtained that it is a normal metabolite in mammals. Growth factors, not identical to known dietary essentials and still unidentified, are apparently necessary for the chick. Convincing proof that chicks require essential fatty acids has been obtained for the first time. The guinea pig is the only animal studied thus far which develops an anemia from deficiency of essential fatty acids. The Sherman diet widely used to produce scurvy has been found deficient in several factors in addition to ascorbic acid. The 5-hydroxy analog of vitamin B_{12} has been found to have effects suggesting that it may be a metabolic antagonist. 2-Amino-2-methyl-1-propanol has been shown to be a choline antagonist in chicks. The compound SKF 525-A, known to inhibit certain metabolic processes, was shown to be capable of decreasing the rat's requirement for certain vitamins. Additional evidence has been obtained that large amounts of dietary vitamin C increase the supply of certain B vitamins in tissues and organs. Experimental diabetes results in inefficient utilization of carotene (provitamin A) under certain conditions, although it was shown that the pancreas is not directly involved in this process. Selenium poisoning has been shown to reduce the utilization of vitamin A and carotene, apparently due to destruction in tissues. The requirements of the guinea

pig for nicotinic acid and folic acid have been defined for the first time. Factor 3, a vitamin-like but still unidentified factor which is necessary to prevent fatal liver necrosis in rats, has been shown to be involved in carbohydrate metabolism. Animals deficient in the factor exhibit impaired glucose utilization, which is rapidly corrected by purified concentrates of Factor 3. This has led to a new, rapid assay for Factor 3 which is independent of vitamin E. Certain antioxidants, but not others, can replace vitamin E and Factor 3. Mice fed Factor 3 deficient diets develop not only liver necrosis but also striking necrotic degeneration in other organs such as heart, peripheral muscle, kidneys and probably the pancreas. Under certain conditions, chicks do not appear to need vitamin E; under other conditions they develop exudative diathesis in the absence of Factor 3. Animals deficient in Factor 3 show a characteristic metabolic defect in liver which seems to involve oxidative phosphorylation and is manifest by a failure in respiration, defective utilization of acetate for fatty acid synthesis, ketone body production and CO_2 formation. Respiratory failure is corrected very quickly by intraportally injected vitamin E and certain antioxidants, but not by Factor 3. The anatomical site of this defect may be in the mitochondria (see microscopy). A new method for the large scale concentration of Factor 3 from natural products has been developed. Ethylene oxide has been shown to cause extensive destruction of certain vitamins when used to sterilize diets. The details of this action are being elucidated since this finding has considerable practical importance in the food industry and in the field of food additives. Enzyme systems have been identified which convert prefolic acid to citrovorum factor. Some of these reactions are inhibited by the antagonist aminopterin. ATP, Mn, citrate and DPN are required for the enzymatic conversion of folic acid to p-aminobenzoic acid and pteridine. Substantial progress has been made in isolating a folic acid metabolite from human urine which appears to be anhydro-citrovorum factor. Methods for the extraction of prefolic acid from liver have been developed.

Minerals - Many mineral ions function in metabolism in several different ways. The mechanical, physicochemical and metabolic factors involved in mineral metabolism are being studied. A theory was developed to explain the differential rates of exchange of two or more co-existing species of ions of the same charge across permselective membranes. The predictions of the theory have been verified by quantitative studies in a variety of systems. These observations could furnish a basis for understanding the preferential accumulation of certain ions by living cells. New types of membranes have been prepared which have great promise in the study of these very complex systems. Radioactive tracers are now being used to elucidate some of these phenomena. Tracers have also been applied to demonstrate that the selective transport of potassium into nerve, and perhaps muscle, cells is related to cellular metabolic activity, whereas the outward passage of sodium was not. The findings and implications of this work are that certain drugs and hormones function directly on cellular permeability whereas others function indirectly by influencing cellular metabolic activity. The very fine technics developed in these studies will be applied to studies of the action of insulin on cellular metabolism. Systematic studies of the safety and toxicity of iodates (as substitutes for iodide in iodized salt)

indicate a substantial margin of safety and lend hope that iodates may be safe for use in situations where iodide is impractical. The long term, large animal toxicity studies necessary for final evaluation of safety in human diets are now under way. Inorganic sulfate has been shown to be capable of replacing dietary methionine to a certain extent in chicks. Certain minerals, namely $FePO_4 \cdot 4H_2O$, $MnSO_4 \cdot H_2O$ and $CuSO_4 \cdot 5H_2O$, have a marked influence on the development of rancidity in diets. K_2HPO_4 accelerates the "browning reaction" in diets.

Hormones - A reproducible insulin bioassay method has been developed which will detect 250 microunits of insulin. Totally depancreatectomized (and hypophysectomized) rats have been found to be very sensitive to insulin, responding to as little as 100 microunits. The possibility that such animals might be used for insulin bioassay is being explored. An improved understanding of the bioassay of prolactin has led to evidence that detectable amounts of this hormone do not occur in human blood or urine, in contrast to previous reports, and hence cast doubt on previous hormonal explanations of gynecomastia in man. Isolation studies are underway to determine whether ACTH and MSH (melanophore stimulating hormone) are indeed separate and distinct hormones. Evidence has been obtained that transection of the midbrain abolishes the pituitary ACTH response to peripheral stress whereas spinal cord transection did not. The adrenal steroid excretion pattern of mice bearing transplantable ACTH producing tumors has been determined. A bioassay method for TSH (thyroid stimulating hormone) has been developed and is being applied in a project to isolate TSH from bovine pituitary substance. A number of new chemical compounds have been produced in the course of studies designed to synthesize a new meta isomer of thyroxine. New insight into the stereochemistry of ephedrine and pseudo-ephedrine has been obtained through a series of chemical reactions. A method has been developed which permits the quantitative isolation and identification of 6 adrenal corticoids from human urine. The method is being applied in a variety of human problems. Significant variations have been found in pregnancy and pregnant diabetics will be studied. Several steroidal substances have been isolated from natural products. One of them, carpesterol, was shown not to be a sterol as believed by previous workers, but a pentacyclic triterpene. New dihydro derivatives of tomatidine and solasodine have been prepared by opening of the F ring. The 26-N-ethyl derivatives of these dihydro derivatives also have been synthesized. This confirms the existence of C_{25} isomerism and the epimeric relationship of solasodine and tomatidine. The anthrasteroid rearrangement described in previous reports has been extended to a wider range of steroids including those occurring in the animal organism. The pathway of t is unusual rearrangement has been established by the isolation and characterization of an important intermediate. The biogenesis of steroids in tumor cells is being studied by synthesizing compounds with ring openings between positions 2 and 3 or 3 and 4 and then determining whether such compounds are used in cholesterol biosynthesis in normal and Ehrlich ascites tumor cells, or whether possible antagonists are formed. The sapogenin precursor, kryptogenin, was studied extensively to settle a disputed point in

the stereochemistry of the side chain. Two new compounds were isolated and proof of their structure is under way. A method was developed to prepare 26-hydroxy-cholesterol from kryptogenin. The former compound appears to be of interest in metabolic studies. A method has been developed to identify as little as 300 µg of steroid hormones using potassium bromide discs and infrared spectroscopy. The aglycone of stevioside (sweet herb of Paraguay) is being studied to establish the steric location of the carboxylic group, the methyl groups and the steric juncture of the four hydroaromatic rings. The steroidal content of human feces is being studied. Sixty fractions, 7 of which are highly purified, have been obtained thus far.

Enzymes - New enzymes too numerous to list have been discovered or isolated in a highly purified state. Most of these are involved in research findings described in other sections of this report. Among these may be mentioned an enzyme which converts a metabolite of 3-hydroxyanthranilic acid to picolinic acid, an enzyme which converts galactose to glucose, one which converts gluconic acid to gluconic acid 6-phosphate, several involved in the "oxidative" pathway of carbohydrate metabolism, others which are involved in the detoxification of the steroidal anesthetic Viadril and in the metabolism of bile acids and sterols, another which catalyzes the phosphorylation of glyceric acid by ATP to 3-phosphoglyceric acid, others necessary to form methylthiol esters of this compound. In many instances these enzymes have been obtained in a highly purified state, assay methods developed, cofactors defined, substrates and products determined and the kinetics and stoichiometry of the reactions elucidated.

Nucleic Acids - There are probably hundreds of these substances in mammalian cells each with a special function in heredity and metabolism. Their chemical structure, methods of synthesis, degradation and conversion are of obvious fundamental importance. New enzymes have been found which synthesize dinucleotides and larger polynucleotides from small molecules. In cooperation with outside scientists the structure of large, enzymatically synthesized polynucleotides has been studied to show that these substances behave enzymatically and chemically exactly like ribonucleic acid except for the distribution of the nitrogenous bases. Perhaps the most significant discovery in this work was the finding of a new class of small ribopolynucleotides which were formed by an enzyme purified from liver nuclei. These new substances contain 5'-phosphomonoester endgroups in contrast to all previously studied related compounds, and appear to be active intermediates in polynucleotide biosynthesis.

Metabolic Studies - The source from which the body draws nitrogen when the diet supplies insufficient protein is of considerable interest. Evidence has been obtained that the greatest part comes from myosin, actomyosin and the water soluble proteins of muscle. Dogs subjected to midbrain transection appear to develop marked arteriolar spasm with reduction in effective glomerular filtration, effective renal plasma flow and cardiac output. Such dogs also show marked changes in creatine and creatinine output. The latter phenomenon is now being studied

with N^{15} labelled tracers. Creatinuria also occurs in thyrotoxicosis. Tracer studies in this disease indicate that the excess urinary creatine arises not from muscle creatine but from freshly synthesized creatine formed chiefly in the liver. Formiminoglycine has received special attention since it has been shown to be metabolized via one carbon intermediates and to require folic acid or related pteridine derivatives as a catalyst. Preliminary evidence indicates the formation of formiminophosphate as a key intermediate. This compound may have a role in metabolism comparable in importance to the carbamylphosphate scheme recently demonstrated by Lipmann. DPN has been shown to be active in reversing the metabolic defect observed in the mitochondria of liver cells from animals deficient in Factor 3.

Photosynthesis and Photo-Action - Biochemical research in this area has been discussed under Carbohydrates. Instruments have been developed which permit detailed short interval observations on the utilization of radiant energy in chemosynthesis and metabolism of photosynthetic unicellular cells. The unique time resolution in these experiments has made possible a number of related, unreported observations which point toward clarification of the sequential role of various metabolic intermediates and related changes in their respective reservoirs. In other studies, newly developed infrared instruments have made possible a study of hydrogen bonding in chemical molecules. Temperature, concentration and the presence of other substances were shown to influence this fundamental phenomenon. The infrared spectra of a number of complex compounds has been determined as part of a project to elucidate the mechanism by which ultraviolet light converts ergosterol to tachysterol (related to parathyroid hormone) and calciferol (vitamin D_3). Conditions have been developed whereby the latter reaction can be studied quantitatively. The flux and charge spectrum of heavy primary nuclei entering the upper atmosphere have been studied. A new cosmic ray phenomenon in which a neutral particle decays with the formation of a pair of positive and negative π-mesons has been observed. Possible biological effects of cosmic rays are being explored in current experiments.

Blood - Dextran and PVP (polyvinylpyrrolidone) have been advocated as "plasma expanders" to substitute for blood or plasma in emergency situations. Study of these substances in animals has revealed some unexpected effects. In dogs and rats, PVP and dextran given intravenously produced an immediate fall in blood pressure, followed by partial recovery, then a continued fall with signs of edema and flushing. This is in contrast to the rise in blood pressure which occurs in other species. Isolated dog or rat limbs perfused with PVP or dextran develop reduced blood flow and edema. Other species were relatively unresponsive. Abnormal human hemoglobins have been studied extensively. It was found that the simultaneous presence of hemoglobins S and D result in an anemia similar to sickle cell anemia; the simultaneous presence of hemoglobins E and Cooley's trait results in anemia similar to Cooley's anemia. Abnormal hemoglobins are involved in some cases of Cooley's anemia; in others, only normal adult and fetal hemoglobin are present. A new abnormal hemoglobin which is not associated with anemia has

been found. All known human hemoglobins have been characterized in a series of univalent buffers to serve as reference standards and to check reports of new hemoglobins. A blood borne substance which stimulates red cell formation has been studied in animals made anemic or subjected to low oxygen tension. Some of the characteristics of this "hematopoetic" factor have been elucidated. Using radioactive tracers it has been found that the longevity of turtle red blood cells exceeds 500 days while that of toads exceeds 270 days. This markedly exceeds the red blood cell life span of warm blooded animals and suggests correlation between length of life and life span of red blood cells in poikilothermic animals. Cobalt did not stimulate red blood cell formation in turtles or toads as it does in warm blooded animals.

Respiration - Special equipment has been devised which permits quantitation of respiratory physiology of human beings swimming under water. The average efficiency of underwater swimming did not exceed 5.1%. At the highest swim rate (1.0 knot) the average work accomplished was 800 ft.-lb./min. This corresponds to light work in air. Personnel who were highly trained maintained a high alveolar CO_2 tension during underwater swimming. For long distance swimming, a rate of 0.7 knots was most efficient physiologically. Respiratory physiology in insects has been studied to show that exposure to low oxygen tensions will speed the first appearance of gas in the respiratory passages of insects which are molting. Insects appear to possess a metabolic process which clears their respiratory passages of liquid which is present before molting.

Analgesics - Pain relieving drugs continue to pose problems of addiction. Drugs with better pain relieving and less addicting properties are badly needed. NIAMD scientists have approached these problems with both clinical and laboratory studies. One phase of the NIAMD program is to gain systematic information correlating chemical structure with analgesic effectiveness and addiction potential. The outstanding observation in this area, made both here and elsewhere, is that substitution of a phenylethyl or closely related structure for methyl on the heterocyclic nitrogen of most types of analgesic compounds enhances analgesic effectiveness and has a variable effect on toxicity and addiction liability. Three new phenylethyl derivatives of meperidine have been prepared by a new method of synthesis. Two of these have about 3 times the analgesic potency of the parent compound. Other phenylethyl derivatives of analgesic agents were prepared which had a greatly lowered analgesic effectiveness. A compendium of available knowledge relating chemical structure to analgesia and addiction has been prepared for the World Health Organization. A compound called piperidyl methadone has been evaluated in patients. As compared to morphine on a weight basis, twice as much of this compound is required for analgesia but no side effects have appeared thus far. Some recent work suggests that glutarimide may be an effective antidote to barbiturate poisoning. A series of glutarimide derivatives has been synthesized for testing. A new series of hydroxycodeinone derivatives has been obtained. A dihydrocodeine derivative with an enlarged nitrogen ring has been synthesized. A new alkaloid, neopine methyl ether has been isolated and its structure proved. Only two of the four possible isomers of cocaine are known. In efforts to synthesize

the two unknown isomers, a method was perfected to synthesize 2-carbomethyoxytropinone, an essential intermediate in the synthesis of the missing allococaine and allopseudococaine. Several compounds related to metapon have been prepared but have shown no promise of practical value as analgesics. A number of compounds chemically related to morphine have been synthesized for structural studies and for testing of analgesic potency and addiction liability.

Shock - The clinical study of oral saline versus plasma therapy of burn shock in man is virtually finished. The first report of this work will be published shortly. Pseudomonas pyemia and septicemia has been found to be a major cause of late death in burns. Chlorpromazine, and a metabolic product (Sulfoxide) have a therapeutic effect in tourniquet shock in mice. Chlorpromazine increases blood volume, lowers plasma proteins and hematocrit, indicating a movement of fluid from extravascular spaces into the blood, and possible clinical usefulness in major surgery. Preliminary findings reported last year have been confirmed and extended, namely, that plasma administered early to shocked mice accumulates largely in the area of injury and is therapeutically ineffective when compared to plasma given later.

Microbes - The quantitative evaluation of drugs in mice infected with leprosy has yielded results closer to human tuberculosis problems, than have mice infected with tuberculosis organisms. The benzaldehyde of isonicotinyl hydrazone had definite suppressive activity in mouse leprosy whereas a closely related derivative and the parent compound, isoniazid, were ineffective in prolonged treatment. Cycloserine was also found to be effective in mouse leprosy. A method has been perfected to produce consistently a type of bacterial endocarditis which resembles human infections. Dogs with surgically damaged heart valves can be infected easily with several Streptococcus and Staphylococcus strains. Diffuse proliferative glomerulonephritis often develops in these animals. Dogs show less valvular heart damage and less susceptibility to endocarditis than rats when exposed to high altitude. Central nervous system lesions comparable to those found in man have been found in mice infected with trypanosomes and treated so as to prolong the disease into late stages. Strains of Streptobacillus moniliformis have been isolated from atypical human infections and from cases of rat-bite fever. These strains have been used to produce acute and chronic lesions in mice and monkeys. A method has been developed to purify hyaluronidase from certain strains of Streptococci. The purified enzyme has been shown to be antigenic in rabbits. Antibody so produced has been used to study tissue localization in sensitized animal tissues using the fluorescent antibody technic.

Microscopy - The electron microscope has been used to trace the development of herpes virus in brain, and to visualize the poliomyelitis virus within brain cells. A number of technical improvements in methods for tissue sectioning and visualization have been made during the year. A projection X-ray microscope has been put into operation and its

applicability to biological problems is being studied. Mice bearing tumors which secrete melanophore stimulating hormone have been shown to develop adrenal hypertrophy, diabetes insipidus and cessation of estrus. Succinic dehydrogenase has been quantitated in renal tubule segments. Mercurial diuresis was shown not to depend on changes in this enzyme. Iodomonoformazan is the best indicator yet developed for detecting this enzyme. Lens protein, pancreatic zymogen, and to a lesser extent pepsinogen, react in a modified Erlich pyrrole reaction. Enterochromaffin did not react and melanin was doubtful. Gastric and intestinal enterochromaffin may differ in the same species as well as from one to another species as judged by histochemical reactions. The yellow pigment (melanin) of guinea pig hair and nerve cell melanin are different histochemically. Numerous methods have been developed or improved to permit precise tissue localization of specific tissue components using histochemical technics. Clear evidence has now been obtained that the subcutaneous fibroblast is the cell of origin of tumors which appear in chickens injected with Rous virus. The muscle lesions which develop in such chickens arise in a characteristic manner which is different from the usual sarcomatous muscle lesions. Central nervous system lesions of dogs are being classified and studied. These lesions are rare and little is known. The existence of osteoarthritis in mice which closely simulates human degenerative joint disease has been established. Animals on diets which produce necrotic liver degeneration have been found to show abnormalities in liver mitochondria long before the necrotic liver syndrome appears. The abnormal mitochondria may disappear or calcify. In some cases the parent cell dies, or in other cases it survives without mitochondria. In such cases, cells still having mitochondria show filamentous forms suggesting a compensation for the lost mitochondria. Regeneration of mitochondria appears to be dependent on the presence of glycogen within the cell. Iodates have been shown to cause damage to the parietal cells of the stomach, and to the retina, when administered or fed in toxic doses.

Fat - The production of dietary obesity, and many of the characteristics of this condition in rats were reported last year. It has now been found that diets supplying 20% by weight of fat are optimal for producing this condition. It may be more than a coincidence that diets with the same fat level also produce the most rapid growth during the period of active body growth. Obese rats fed a stock diet, or the obesity producing diet in reduced amounts, seem to live longer than rats whose weight is not reduced. Some of the obese rats show joint changes suggestive of osteoarthritis. An interesting association between obesity and osteoarthritis has also been observed in mice. One strain (STR/1N) develops obesity and in about 93% of such mice osteoarthritis is found. In other closely related strains in which obesity does not develop, only about 2% show osteoarthritis. C57 mice develop a moderate obesity when fed a 60% fat diet.

Diagnostic Tests - A specific enzyme test for galactose diabetes was mentioned under Carbohydrates. In addition, considerable progress has been made in studying a specific serologic test for rheumatoid arthritis. The test appears to depend on the presence of an inhibitor (to red cell agglutination) in normal serum which is absent in the serum of patients with rheumatoid arthritis. Glycyl glycine, ureidosuccinic acid, 5-acetic acid hydantoin and glucosamine show some "inhibitor" activity. These compounds or their metabolic products may act by competitive binding to a compound of the test system. Progress has been made in developing methods to purify from urine a substance probably related to isoglutamine which is excreted by folic acid deficient rats. Assays for this substance as a test for folic acid nutrition may become a useful diagnostic test if assay methods can be perfected.

Other Research Functions - Several groups in NIAMD conduct laboratory operations which in themselves are usually not research, but which are vital to the research program of this and other Institutes. One group does microanalytical determinations of various elements and substances such as carbon, hydrogen, nitrogen, phosphorous, reducing sugar, halogens, lactic acid, methoxyl, acetyl, carboxyl, sodium, potassium, iron, barium, antimony and other substances. Approximately ten thousand such analyses were performed during the year. Infra red spectra, an increasingly important method of identification of organic compounds, were obtained on many compounds. Another group performs quantitative amino acid and vitamin analyses on food materials, special diets, urine, blood, tissues and other biological materials. Another group does the gross and microscopic pathology on monkeys used in safety testing of poliomyelitis vaccine, a very considerable task during the past year. Others do gross and microscopic pathology on animals and on human beings from a variety of clinical and laboratory research activities.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD
INSTITUTE

2. Biochemistry and Nutrition
[illegible]

3. Biochemistry and Physiology of Nutrition
SECTION

4. _____
LOCATION

5. NIAMD-1
SERIAL NO.

6. Vitamin Metabolism
PROJECT TITLE

7. F. S. Daft
PRINCIPAL INVESTIGATOR(S)

8. E. G. McDaniel, M. Martin and M. L. Kunde
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

A. To discover substances which decrease vitamin requirements and to elucidate their mechanism of action.

B. To define the functions of amino acids and nucleic acids in folic acid metabolism.

Methods: Purified diets with or without specific vitamins, and with or without substances which may "spare" vitamin requirements are fed to animals. Growth and development, physical signs and symptoms, blood changes, tissue concentration and urinary excretion of vitamins and metabolites are studied.

Major Findings:

1. Previous work has shown that large amounts of dietary vitamin C or small amounts of certain antibiotics have the ability to decrease markedly the rat's requirement for a variety of B vitamins. Recent work has shown also that this ability is shared by certain compounds known to inhibit certain metabolic processes in mammals, such as the compound called SKF 525-A.

2. Penicillin administered parenterally finds its way into the gastrointestinal tract via the bile. Technics have been developed to divert the biliary secretions from the intestinal tract as an approach to determining whether antibiotics exert their vitamin sparing effect in the G-I tract or in the tissues. Other studies have shown that whatever the mechanism may be, large amounts of dietary vitamin C result in an increased supply of certain B vitamins in tissues.

3. Previous work has shown that folic acid deficient rats excrete large amounts of urinary forminino glutamic acid and that this compound arises from the amino acid histidine. Further work has shown that histidine, tryptophane or threonine, or certain nucleic acid derivatives counteract partially the effects of folic acid deficiency.

Significance to NIAMD Research: This basic research may have considerable significance in elucidating the various factors which influence vitamin requirements, throw considerable light on vitamin metabolism and offers some hope of providing diagnostic tests for specific vitamin deficiencies in man and animals.

Proposed Course: Current work is directed toward elucidating the mechanisms of these effects using various routes of administration of active substances, vitamin balance techniques and dietary manipulations.

Analysis of NIH Program Activities

Budget Data and Publications Sheet

10. NIAMD-1
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. COOPERATING UNITS:

Dr. M. Silverman (Section on Isolation and Fractionation)

14. No parallel research in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Briggs, G. M. and F. S. Daft. Water-Soluble Vitamins, Part I. (Vitamin B_{12}, Folic Acid, Choline and Para-Aminobenzoic Acid). Ann. Review of Biochemistry, Vol. 24, 339-392, 1955.

17. No honors and awards.

Analysis of NIH PROGRAM ACTIVITIES

Project Description Sheet

1. NIAMD
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY

3. Biochemistry and Physiology of Nutrition
SECTION

4. _____
LOCATION

5. NIAMD-2
SERIAL NO.

6. Nutritional Aspects of Growth and Longevity
PROJECT TITLE

7. J. M. Hundley
PRINCIPAL INVESTIGATOR(S)

8. R. B. Ing
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To relate various rates of growth to body composition, to the development of disease, and to longevity.

Methods: Various diets are fed to rats to produce planes of growth varying from severe retardation to "above normal". After active grow th has ceased, a uniform diet is fed to certain groups so that possible dietary influences will be uniform except during the active growth period. Two strains of rats are used: one is naturally slow growing and has attained mature body weights of 600-900 gms.; the other strain exhibits rapid growth, tends to become obese, and attains mature weights as high as 1400 gms.

Results: This study is a long term project which will require two additional years to complete. No major findings have developed thus far.

The diets selected for the study have yielded the various planes of growth desired. Body composition studies have confirmed the fact that the various diets have influenced not only the % body fat but have produced desired differences in "lean body mass" growth.

A simple, reasonably accurate method has been developed to determine total body fat using specific gravity technics.

Significance to NIAMD Research: Numerous studies have revealed the fact that American children are growing at constantly faster rates. It is not known whether this is a desirable trend with respect to eventual adult health, longevity, and chronic disease prevention. This animal study offers an approach to this problem since the experiment is designed to make growth rates during "childhood" the only variable.

Proposed Course: To be continued as presently conducted until all or substantially all of the animals have died as the result of natural causes. Causes of death are being determined and studied.

Analysis of NIH Program Activities

Budget Data and Publications Sheet

10. NIAMD-2
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S)

None

14. No parallel research in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

"Height and Weight of First-Grade Children as a Potential Index of Nutritional Status". J. M. Hundley, O. Mickelsen, N. Mantel, R. N. Weaver, and R. C. Taber, Am. J. Pub. Health 45: 1454 Nov. (1955).

"Need for Weight Control Programs". J. M. Hundley, Weight Control, Iowa State College Press, pp. 1-17 (1955).

"Summary", Weight Control, ibid, pp. 231-238.

"Weight Control and Health". J. M. Hundley, Food and Nutrition News 27: No. 2, Nov. (1955).

17. No honors and awards.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY

3. Biochemistry and Physiology of Nutrition
SECTION

4. _____
LOCATION

5. NIAMD-3
SERIAL NO.

6. Nutritional Improvement of Cereals with Amino Acids
PROJECT TITLE

7. J. M. Hundley
PRINCIPAL INVESTIGATOR(S)

8. R. B. Ing, Gordon Sampson (C. C.) and G. Donald Whedon (C. I.)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: (a) To determine whether supplements of lysine, threonine and other amino acids will improve the biological value of rice protein in man. This is an extension to man of animal research findings (b) To find rich but economical sources of threonine in foods (c) To determine the value of amino acid and other nutrient supplements with cereal proteins other than rice.

Methods: In man, the nitrogen balance technic is used to assess the value of amino acid supplements in rice diets. In animals, growth, food efficiency, and protein efficiency are used as indices of the value of various supplements.

Major Findings: 1. Supplements of lysine and threonine have been shown to cause a positive shift in nitrogen balance in some subjects but not in others. In some subjects, lysine, threonine plus methionine were effective where lysine and threonine alone were ineffective. The significant and unexpected finding in these studies is that in one subject lysine and threonine had a negative effect on nitrogen balance. Furthermore, a mixture of the 8 essential amino acids usually resulted in a positive nitrogen balance, but in on subject caused a definite negative response. These findings constitute strong support for the importance of "amino acid balance" in nutrition and demonstrate how an upset in

this balance may be produced by relatively small additions of amino acids under circumstances where there is every reason to expect a favorable response.

2. Two algae, Scenedesmus obliquus and Chlorella pyridenosa, have been shown to be unusually good sources of threonine as supplements to white flour and white bread diets fed to rats. These algae compare very favorably to high quality proteins such as egg and milk as a source of this amino acid.

3. For the first time, unequivocal evidence has been obtained that the vitamin-mineral enrichment formula used in white bread does improve the nutritional value of such bread when fed as a sole food source to animals.

Significance to NIAMD Research: The importance of the amino acid supplement studies relates to the growing interest in adding synthetic amino acids to various protein foods to improve their biological value. Since quantitative and qualitative protein deficiencies are a major health problem in many areas of the world, these studies may have some practical importance.

Proposed Course: The clinical studies will be continued to further elucidate some of the unexpected phenomena referred to above. The animal studies have been concluded and are being prepared for publication.

Analysis of NIH Program Activities

Budget Data and Publications Sheet

10. NIAMD-3
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S)

NIAMD Clinical Investigations Branch (Dr. Whedon)

The Clinical Center (Gordon Sampson)

University of Maryland (Dr. R. W. Krauss)

14. No parallel research in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

"Nutritional Needs of Institutionalized Patients". J. M. Hundley, Am. J. Pub. Health 45: 328 Mar. (1955).

"Niacin and Anti-Niacin Activity of 3-Acetylpyridine in Dogs". E. G. McDaniel, W. H. Sebrell and J. M. Hundley, J. Nutrition 55: 623 Apr. (1955).

"Nutrition Practices: A Guide for Public Health Administrators". J. M. Hundley (with a committee of several others), Am. Pub. Health Assoc., Inc. 1790 Broadway, New York 19, N. Y. (1955).

17. No honors and awards.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY

3. Biochemistry and Physiology of Nutrition
SECTION

4. _____
LOCATION

5. NIAMD-4
SERIAL NO.

6. Diabetes and Niacin Metabolism Studies
PROJECT TITLE

7. E. G. McDaniel, J. M. Hundley, W. H. Sebrell
PRINCIPAL INVESTIGATOR(S)

8. Alan Mehler, Sidney Chernick
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Objectives: To study the metabolism of the amino acid tryptophan, and the formation of the B vitamin niacin from tryptophan in normal and diabetic animals, and to determine what corrective measures may be taken with respect to metabolic defects observed.

Methods: It has been shown that in the alloxan diabetic rat there is a marked reduction in the conversion of tryptophan to niacin. The site(s) of this metabolic defect are being studied. The effects of insulin, adrenalectomy and adrenal hormones on this conversion are investigated. By use of chemical, chromatographic and microbiological procedures the effect of diabetes upon the amounts of the various metabolites formed from tryptophan are studied. With Dr. Alan Mehler and Dr. Sidney Chernick quantitative studies of enzymatic reactions involved in the conversion of tryptophan to niacin are in progress.

Experiments are done to determine whether a similar defect is observed in other animals (including man) and in other types of diabetes.

Major Findings: The conversion of tryptophan to niacin is greatly reduced in diabetic rats. This conversion was restored to normal by administration of insulin and tryptophan for sufficiently long periods. Conversion of tryptophan to anthranilic acid and 3-hydroxyanthranilic acid was also greatly reduced, while conversion to xanthurenic acid was markedly increased in diabetic rats.

Experiments with Dr. Mehler demonstrate that alloxan diabetes result in a marked increase in a liver enzyme which is responsible for the conversion of 3-hydroxyanthranilic acid to picolinic acid (another metabolite of tryptophan). The level of this enzyme could be reduced to normal by administration of insulin. Adrenalectomy also resulted in restoration to normal of both the conversion of tryptophan to niacin, and the level of the above liver enzyme.

Significance to NIAMD Research: In view of the large number of human diabetics throughout the world, the results of a study of this type could be of primary importance. Numerous complications other than abnormal glucose utilization are known to be associated with diabetes in humans. It is possible that some of these complications may result directly or indirectly from abnormal metabolism of proteins, amino acids or vitamins, or from an inability to utilize in a normal manner some of these factors.

Proposed Course: Studies are now in progress to determine the sites of other defects in the metabolism of tryptophan. Both liver enzymes (Dr. Mehler) and blood enzymes (Dr. Chernick) are being studied. Experiments are in progress to determine the manner in which insulin restores the ability of the diabetic rat to make the conversion, and to determine whether or not tryptophan or its metabolites are necessary to obtain a favorable response to insulin.

The manner in which adrenalectomy corrects the defect is also under investigation, as well as the effects of adrenalectomy or administration of adrenal hormones.

Experiments will be done in an effort to determine the source of the blood glucose which was found to be elevated following administration of tryptophan to diabetic rats, and to determine the relationship, if any, between blood glucose levels and the metabolism of tryptophan. The possible role of pyridoxine in these phenomena will be investigated.

B. Objectives: To study the development or prevention of niacin deficiency in animals fed large amounts of corn.

NIAMD-4
SERIAL NO.

Methods: Experiments are being done to determine whether or not alkaline treatment of corn (similar to that used in the preparation of corn for human consumption in some areas of the world) has any beneficial effect in the prevention of niacin deficiency in dogs and rats.

Major Findings: Preliminary experiments indicated that dogs fed high corn diets were able to obtain more niacin from alkali treated corn than from untreated corn, as judged by N'-methylnicotinamide urinary excretion.

We have succeeded in decreasing the time necessary to develop niacin deficiency (blacktongue) in dogs fed corn diets, by substituting an equal amount of gelatin for the cow peas originally used in the Goldberger diet.

Significance to NIAMD Research: In some parts of the world there are still large populations for whom a large part of the normal diet is corn. Since it is known that diets consisting chiefly of corn are inadequate with respect to prevention of niacin deficiency, it is of importance to determine whether or not the availability of niacin may be changed by food preparation methods.

Proposed Course: The effect of alkali treatment of corn will be tested with respect to its ability to prevent development of blacktongue, to alter the urinary excretion of niacin metabolites in dogs, and to effect growth of rats. Experiments will be done to determine the manner by which availability of the niacin is increased by such treatment.

Analysis of NIH Program Activities

Budget Data and Publications Sheet

10. NIAMD-4
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S)

Alan Mehler NIAMD, L.P.B.

14. No parallel research done in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

"Niacin and Anti-Niacin Activity of 3-Acetylpyridine in Dogs".
E. G. McDaniel, W. H. Sebrell and J. M. Hundley, J. Nutrition 55: 623 Apr. (1955).

17. No honors and awards.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases — INSTITUTE
2. Biochemistry and Nutrition — LABORATORY OR BRANCH
3. Section on Biochemistry and Physiology of Nutrition — SECTION
4. — LOCATION
5. NIAMD-5 — SERIAL NO.

6. Nutritional significance of vitamin B_{12} and related compounds for the chick and factors influencing the stability of the diets employed — PROJECT TITLE

7. Dr. M. R. Spivey Fox — PRINCIPAL INVESTIGATOR(S)

8. Dr. George M. Briggs, Ligia O. Ortiz, Dr. Olaf Mickelsen, Dr. Richard Yamamoto, Susie N. Hagan — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: To determine the biological function and activity of vitamin B_{12} and related compounds in the chick. To determine factors that cause deterioration of the experimental diets used in these studies.

Methods Employed: Deficiencies of vitamin B_{12}, methionine, and choline have been produced in the young chick by their omission from the diet and the use of specific antagonists. The effect of other nutrients in increasing or decreasing the severity of these deficiencies has been studied. The activity of some structural analogs of vitamin B_{12} has been determined. The factors affecting development of rancidity and browning in diets have been followed by chemical, olfactory, and feeding tests.

Major Findings: (1) Chicks fed diets ranging in vitamin B_{12} content between 5 γ and 100 γ/kg. of diet stored approximately 10% of the intake in the liver. At higher levels the percentage stored was markedly lower.

(2) Addition of intrinsic factor to the vitamin B_{12}-free diet did not alter growth or B_{12} concentration in the liver. Intrinsic factor did reduce growth in some instances, as well as lower the amount of vitamin B_{12} stored in the liver of the chick receiving optimal levels of B_{12} in the diet.

(3) There are still a number of compounds chemically related to vitamin B_{12} whose activity in chicks has not been determined. The chick is an important animal for vitamin B_{12} studies since it

shows such consistent evidence of a dietary deficiency. Desdimethyl vitamin B_{12} and the 5-hydroxy analog of B_{12} each had about 10% of the activity of vitamin B_{12}. The 5-hydroxy analog has depressed growth of chicks in some experiments where it was injected equivalent to 10 γ/kg. of diet. This possible antagonistic action is to be explored further. The activities of acetatocobalamin and sulfatocobalamin were comparable to vitamin B_{12}. Chlorocobalamin and nitrocobalamin appear to be somewhat more active than vitamin B_{12} when fed at 10 γ/kg. of diet. When injected, or fed at higher levels, the activities were similar to that of B_{12}. Acetato-, chloro-, nitro-, and sulfato- cobalamins were stored in the liver in amounts comparable to that of vitamin B_{12}. Factor B, a substance showing activity in one microbiological assay, had no vitamin B_{12} activity in the chick.

(4) No histological abnormalities have been detected in the vitamin B_{12} deficient chicks.

(5) 2-amino-2-methyl-1-propanol, which has been shown to antagonize choline in the rat, caused depression of growth and perosis in chicks when incorporated into a variety of diets. These adverse effects were overcome with additional choline. This antagonist is particularly advantageous in experiments, such as the vitamin B_{12} studies, where diets containing crude materials, and thus choline, must be used.

(6) Chick Salts A and Wesson Salts were far more active in accelerating the development of rancidity in diets than was the Hubbel, Mendel, and Wakeman Salt mixture. The most active individual constituent salts (in decreasing order) were $FePO_4 \cdot 4H_2O$, $MnSO_4 \cdot H_2O$, and $CuSO_4 \cdot 5H_2O$. The relative activity of the complete salts mixtures cannot be explained on the basis of their content of individually active components. Rancidity developed more rapidly with sucrose than with glucose. Mixtures of salts with corn oil became very viscous following the onset of rancidity. Again, Chick Salts A and Wesson Salts were move active than Hubbel, Mendel, and Wakeman Salts.

(7) Rats fed aged diets containing Wesson Salts grew very poorly over a 6-week period, whereas rats fed a similarly aged diet containing Hubbel, Mendel, and Wakeman Salts grew as well as controls receiving fresh diet.

(8) Chick Salts A markedly accelerated the "browning reaction" between casein and glucose. Most of this activity was due to K_2HPO_4, which is not present in Wesson or Hubbel, Mendel, and Wakeman Salts.

Significance to NIAMD Research: There is at present no animal other than the chick in which a vitamin B_{12} deficiency can be produced consistently by dietary means. There is accumulating evidence that the microbiological assays measure substances that are inactive in man. For this

reason it is advisable to evaluate the activity of a variety of compounds related to vitamin B_{12} as far as the chick assay is concerned. Since the chick is such an ideal animal in which to produce a vitamin B_{12} deficiency, it should provide an excellent opportunity for the study of the role of vitamin B_{12} in metabolsim.

The studies on rancidity are of nutritional importance and perhaps of some public health significance since even before rancidity of the fat is apparent by olfactory tests, there are suggestions that vitamin losses have occurred as a result of the incipient rancidity. There is a possibility that some nutritional studies have been complicated by the dietary vitamin losses resulting from the rancid diet.

Proposed Course of Project: To continue along the same general lines with emphasis on determining the function of vitamin B_{12} and the relationship of other nutrients.

Analysis of NIH Program Activities

Budget Data and
Honors, Awards, and Publications Sheet

10. NIAMD-5
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

Laboratory of Pathology, NCI
Arthritis and Rheumatism Branch, NIAMD
Laboratory of Tropical Diseases, NMI
Laboratory of Pathology and Histochemistry, NIAMD

14. No parallel research in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Fox, M. R. Spivey, L. O. Ortiz, and G. M. Briggs. Toxicity of ethionine in the young chick. J. Agr. Food Chem. 3: 436, 1955.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases — INSTITUTE
2. Biochemistry and Nutrition — LABORATORY OR BRANCH
3. Section on Biochemistry and Physiology of Nutrition — SECTION
4. _____ — LOCATION
5. NIAMD-6 — SERIAL NO.

6. Nutritional Studies With the Guinea Pig — PROJECT TITLE

7. Dr. Mary E. Reid — PRINCIPAL INVESTIGATOR(S)

8. Dr. G. M. Briggs, Mary G. Martin, Dr. J. G. Bieri — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: To determine (a) deficiency effects of different nutrients, (b) the quantitative requirement of such nutrients, and (c) to study their physiological action and interrelation with other nutrients by use of the guinea pig.

Methods Employed: Guinea pigs at 2 to 5 days of age, weighing approximately 100 g. were placed on specially prepared experimental diets, complete in all known nutritional factors except the one under investigations. The effect of dietary variation was studied by measurement of weekly weight changes and clinical and biochemical changes in the animal and its tissues, including study of the blood picture.

Major Findings: For the first time definite values were established for the following nutrients required by the guinea pig:

Folic acid: Three mg. per kg. of diet were needed for normal levels of erythrocytes and hemoglobin, but 6 mg. were needed for normal leucocyte levels. The guinea pig appears to be unique in responding to oral leucovorin at levels equivalent to folic acid.

Niacin: Ten mg. per kg. of diet were needed for normal growth on a casein diet, while 20 to 50 mg. were needed on a soy protein (Drackett) diet. The high levels of niacin needed in the soy protein diet are probably associated with its low tryptophan content. Tryptophan can substitute for niacin as far as growth is concerned.

Amino acids: In the absence of dietary niacin the tryptophan requirement for growth is 0.45%; with a deficient level of niacin, the requirement is 0.2% or less. Extra (1%) arginine is required to produce as good growth on 20% casein as on 30% diets. With 20%

Drackett protein, additional arginine is unnecessary but additional methionine is required. Guinea pigs fed for a period of 100 days on a diet devoid of protein but containing amino acids proportioned to the amounts contained in Drackett protein have gained at a rate of approximately 4.5 g. per day as compared to a gain of 6.0 g. in the control animals.

Fat studies: 1% of corn oil in the diet permitted almost maximum growth but did not give full protection against dermatitis, particularly in male animals. A 3% level was adequate for the prevention of dermatitis. Linoleic acid fed (as the urea complex) in the amount contained in 1% corn oil permitted a growth rate close to maximal. The animals have remained in good health and free from scaliness for a period of 250 days. Results suggest that intact fat as such may not be a dietary essential for the guinea pig. Regarding the relation of fat to the blood picture, the guinea pig appears to be the only animal thus far studied which shows an alteration in the blood cells in the absence of essential fatty acids. As compared to control animals, the deficient guinea pigs show lowered hemoglobin and hematocrit values but no change in the number of erythrocytes. Total leucocytes and granulocytes increase in the deficiency.

Development of a practical scorbutigenic diet: The Sherman et al. scorbutigenic diet which has been used extensively for vitamin C studies has been found deficient in factors other than ascorbic acid. Modifications have been made in the diet to correct this condition by the addition of folic acid, calcium carbonate, and ferric citrate.

Other studies: Studies in progress confirm the need for a source of cellulose in the diet of the guinea pig. Other studies indicate the need for an unidentified growth factor in alfalfa which spares the cellulose requirement (other than cellulose per se).

Significance to NIAMD Research: A more complete knowledge of guinea pig nutrition is important to the study of metabolic diseases because, with the exception of the monkey, the guinea pig is in some respects more like man than any other experimental animal. Since it is now possible to rear these animals on a purified diet, they can be used for studying factors responsible for (1) abnormal mineral metabolism such as in certain forms of arthritis and other bone diseases; (2) degeneration of tissues such as dystrophy of muscles as observed in choline, vitamin E, and potassium deficiencies; (3) imbalances in enzyme and hormone production such as the elevated xanthine oxidase in the livers of vitamin E deficient animals, elevated choline esterase in vitamin B_6 deficiency, elevated Co-A enzyme in vitamin B_{12} deficiency; (4) imbalances in the production of blood cells by variations in dietary vitamin C, B_{12}, niacin, and folic acid; (5) the injurious effect upon the gastrointestinal tract caused by the lack of certain vitamins such as B_6 and folic acid; and (6) unidentified growth factors.

NIAMD-6
SERIAL NO.

Proposed Course of Project: Studies on folic acid (citrovorum factor) cellulose, and unidentified factors will be continued. Also, further tests will be conducted on the requirement of the guinea pig for thiamine and niacin. The present studies will be extended to determine the requirement of the guinea pig for pyridoxine, vitamin B_{12}, riboflavin, and essential amino acids. The interrelations of amino acids and of vitamins and minerals in the nutrition of the guinea pig will also be investigated. An attempt will be made to gain additional evidence as to whether or not maximum growth is necessarily a satisfactory criterion of good nutrition.

Analysis of NIH Program Activities

Budget Data and
Honors, Awards, and Publications Sheet

10. NIAMD-6
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. No cooperating units.

14. No parallel research in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Reid, M. E. Nutritional studies with the guinea pig. III. Choline. J. Nutr. 56: 215, 1955.

Reid, M. E., M. G. Martin, and G. M. Briggs. Nutritional studies with the guinea pig. IV. Folic Acid. J. Nutr. (in press).

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases — INSTITUTE
2. Biochemistry and Nutrition — LABORATORY OR BRANCH
3. Section on Biochemistry and Physiology of Nutrition — SECTION
4. _____ — LOCATION
5. NIAMD-7 — SERIAL NO.
6. Studies on the nutrition of the chick — PROJECT TITLE
7. Dr. G. M. Briggs — PRINCIPAL INVESTIGATOR(S)
8. Dr. M. R. Spivey Fox, Ligia O. Ortiz, and Dr. J. G. Bieri — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION:

Objectives: To gain more information of a fundamental nature on the biochemistry, physiology, and nutrition of unidentified factors, essential fatty acids, vitamin E, folic acid, amino acids, and other nutrients in the nutrition of the chick so that this information may be compared with the nutrition of other laboratory animals with eventual application to man.

Methods Employed: By the use of specially prepared highly purified diets composed of all known nutrients (except the nutrient being studied) groups of young chicks are reared in wire-floored cages under standard controlled conditions. The effect of dietary variation is studied by measurement of weight changes, food consumption, and determination of clinical and biochemical changes in the animal and its tissues.

Major Findings: (1) Evidence has been obtained for the need of an unidentified growth-promoting factor for the chick. The factor (or factors) is present in soybean meal, liver, alfalfa, egg, and yeast, and appears to be organic (is not present in the ash of soybean meal). The assay for the factor is based on studies with diets containing sucrose and purified soybean protein. About 80 grams difference in growth at 4 weeks is obtained with the factor. The relationship of this factor to those reported by others is as yet unknown.

(2) Convincing proof that chicks require a dietary source of essential fatty acids has been demonstrated for the first time. Deficient chicks show reduced growth (after 8-12 weeks), a scaly skin, delayed development of sexual maturity, and changes in the fatty acid composition of the blood (marked decrease in linoleic and linolenic acids and an increase in arachidonic acid). Preliminary evidence shows these symptoms to be prevented by dietary methyl linoleate.

(3) It has been found that, under certain dietary conditions, chicks will grow normally (up to 16 weeks at least) without any vitamin E in the diet. This finding is important because it puts new light on the function of vitamin E in nutrition.

(4) Under the conditions published by Dr. M. E. Scott of Cornell, the chick appears to require a source of vitamin E or "Factor 3" in the diet for normal growth and survival. However, under other conditions (with diets containing extracted soybean protein and low vitamin E) no requirement for "Factor 3" has been seen as yet. Thus, the requirement for "Factor 3" in the chick may be related to the presence of "stress" factors in the diet. Further study is necessary.

(5) Additional studies on vitamin A showed that destruction of this vitamin occurred in the diet in the presence of bentonite (a clay used in the pelleting of commercial feeds). Bentonite was not detrimental when added to natural diets or to diets containing "stabilized" vitamin A, unless very high levels (20% or more) were used.

(6) A two week assay for folic acid activity was developed with the chick. Samples containing as little as 100 micrograms of active material could be measured for animal activity by this method.

(7) By using several approaches in repeated experiments no dietary requirement for thioctic acid or molybdenum could be found in the chick in spite of the reported need for these substances.

(8) Marked deficiencies of vitamin K were produced in chicks fed corn-soybean diets, especially in the presence of the drug sulfaquinoxaline, confirming the reports of others.

(9) It has been found, in cooperation with other laboratories, that dietary inorganic sulfur (as sulfates) may spare the chick's requirement for methionine under certain conditions.

Significance to NIAMD Research: The project provides basic information essential to the complete understanding of nutritional and biochemical relationships in metabolism and disease.

Proposed Course of Project: Emphasis this next year will be devoted to gaining more information about the unidentified growth factor(s) for the chick and its relationship to other nutrients. Studies will also be made on the function, biochemistry, and requirements of vitamin E, essential fatty acids, folic acid, and certain other nutrients.

Analysis of NIH Program Activities

Budget Data and
Honors, Awards, and Publications Sheet

10. NIAMD-7
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S). (ITEM 10)

Section on Fractionation & Isolation, LBN, NIAMD
Cancer Chemotherapy National Service Center - NCI

14. No parallel research in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Briggs, G. M., and F. S. Daft. Water-Soluble Vitamins, Part I. (Vitamin B_{12}, Folic Acid, Choline, and Para-Aminobenzoic Acid). Annual Review of Biochemistry, Vol. 24, 339-392, 1955.

Briggs, G. M., and M. P. Spivey Fox. Comparison of Vitamin B_{12} and Desdimethyl B_{12} Activity in the Chick. Proc. Soc. Exp. Biol. Med. 89, 318-319, 1955.

Briggs, G. M., and M. P. Spivey Fox. Vitamin A Deficiency in Chicks Produced by Adding High Levels of Bentonite to Synthetic Diets. Poultry Science (in press).

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases — INSTITUTE
2. Biochemistry and Nutrition — LABORATORY OR BRANCH
3. Section on Biochemistry and Physiology of Nutrition — SECTION
4. _____ — LOCATION
5. NIAMD-8 — SERIAL NO.

6. Effect of hormones on carbohydrate metabolism and on muscle and pelt proteins.
PROJECT TITLE

7. Dr. R. O. Scow
PRINCIPAL INVESTIGATOR(S)

8. Susie N. Hagan
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: To determine the influence of hormones on carbohydrate metabolism and on the proteins in muscles, pelt, and carcass of experimental animals. Also to study the protein fractions and fat content of the thigh muscle in rats of different ages when food is completely withdrawn for varying lengths of time.

Methods Employed: Various endocrine glands are removed from young experimental animals (rats, mice, guinea pigs, toads). Some of these animals are treated with a variety of hormone preparations or are fasted for a given length of time. At the end of the experiment the animals are killed and appropriate measurements are made. Selected muscles are rapidly excised, frozen in carbon dioxide, powdered, and then extracted with a salt solution. Purified protein fractions are prepared by precipitation procedures. Pelt proteins are extracted using a variety of eluting agents. The carcass is dehydrated, defatted, and then a nitrogen determination made to obtain information on the total water, fat, and nitrogen content of the carcass. Mixtures of muscle proteins are separated at a given pH by paper electrophoresis.

Major Findings: During fasting, the greatest percent of nitrogenous material lost comes from the myosin and actomyosin (contractile) and water-soluble proteins.

Testosterone propionate, when administered to gonadectomized rats, caused the usual change in secondary sexual [illegible] tissue but no significant change in the quantity of muscle proteins.

In the hypophysectomized rats treated simultaneously with growth hormone and testosterone propionate, an increase caused by a summation of the effects of the two hormones may be seen in the collagen of both the thigh muscle and the pelt. Myosin and actomyosin are not changed by the hormones whether given alone or in combination.

The protein, tropomyosin, which was previously isolated from rabbit and fish skeletal muscle by other workers, has been prepared from rat thigh muscle.

Electrophoretic studies of the alkali soluble proteins (those not extracted with salt solution) indicate that this fraction consists of tropomyosin and denatured myosin.

During the past year Dr. Scow has been working with Dr. Bernardo Houssay in Buenos Aires. Much of his work there has been with hypophysectomized and pancreatectomized rats. These animals require insulin, otherwise they go into coma within 24 hours after feeding, with 30% of the comatose animals dying. The blood sugar in these animals is about 400 mg. %. The subcutaneous injection of 0.1 milliunits of insulin into these rats that have been fasted for 17 hours produces a significant decrease in the blood sugar. Further work is being done to develop this procedure as an assay for insulin. Other studies are being made to determine the effect of insulin and growth hormone injected into the hypophysectomized-pancreatectomized rats on nitrogen balance and storage.

Significance to NIAMD Research: Since a number of metabolic diseases are associated with marked disturbances of body protein formation, it is important to know the factors that influence muscle growth and formation. Disturbances in the growth of collagen, one of the proteins studied in this project, occurs in arthritis and in a number of other diseases.

The development of an assay for insulin would provide an invaluable tool for the study of diabetes.

Proposed Course of Project: Muscle, pelt, and carcass analyses will be performed on hypophysectomized rats treated with growth hormone, testosterone, or both, and on untreated control animals. The influence of testosterone on the muscle protein of gonadectomized rats will be studied. Studies will be made on the effects of fasting, both the complete withdrawal of food and semi-starvation, on the changes in muscle protein composition.

Analysis of NIH Program Activities

Budget Data and
Honors, Awards, and Publications Sheet

10. NIAMD-8
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S). (ITEM 10)

Endocrinology Branch, NCI
Laboratory of Oral and Biological Chemistry, NIDR
Instituto de Biologia y Medicina Experimental, Buenos Aires, Argentina

14. No parallel research in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Scow, R. O., and M. A. Greer. Effect on the thyroid gland of experimental alteration of the level of circulating thyroxine in mice with heterotopic pituitaries. Endocrinology 56: 590, 1955.

Scow, R. O., and S. N. Hagan. Effect of testosterone propionate on myosin, collagen, and other protein fractions in striated muscles of gonadectomized male guinea pigs. Am. J. Phys. 180: 31, 1955.

Wollman, S. H., and R. O. Scow. Comparison of thyroid lobes, autotransplanted and in situ, in the rat; Growth and I^{131} uptake. J. Nat. Cancer Inst. 15: 943, 1955.

Wollman, S. H., and R. O. Scow. Effect of propylthiouracil on the ratio of the radioiodide concentrations in thyroid gland and serum in normal and hypophysectomiezed rats. Endocrinology 56: 445, 1955.

Wollman S. H., and R. O. Scow. Effect of various goitrogens and of dose of propylthiouracil on the ratio of radioiodide concentrations in the thyroid gland and serum in mice. Endocrinology 56: 448, 1955.

Zipkin, I., and R. O. Scow. Fluoride deposition in different segments of the tibia of young growing rats. Am. J. Phys. (In press).

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Guggenheim Fellowship - Dr. R. O. Scow
Schering Award of The Endocrine Society - Dr. R. O. Scow

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases — INSTITUTE
2. Biochemistry and Nutrition — LABORATORY OR BRANCH
3. Section on Biochemistry and Physiology of Nutrition — SECTION
4. _____ — LOCATION
5. NIAMD-9 — SERIAL NO.

6. Studies on the Nutrition of the Mouse
PROJECT TITLE

7. Dr. G. M. Briggs
PRINCIPAL INVESTIGATOR(S)

8. C. E. Emery and D. Ingle
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: To obtain fundamental knowledge on the nutrition of the mouse and to compare nutritional findings obtained for other laboratory species with those found in the mouse.

Methods Employed: Different genetic strains of mice are weaned as young as possible (12-16 days) and fed various experimental diets including those designed for other species. A total of approximately 8 groups (about 48 animals) are started on experiment on alternate weeks and are continued for 6 weeks or longer.

Major Findings: (1) Several different semi-synthetic diets containing all known growth factors have been developed which result in normal growth and development in mice.

(2) It has been found for the first time that mice fed diets containing large amounts of fat (60 percent or more) do not grow at a maximum rate unless the casein content is increased to 30 percent and unless 5 percent dried whole liver and small amounts of antibiotics are fed. Mice (C57 strain) fed such a fortified diet develop obesity after several months but not to the same extent as rats fed the same diet in studies made by Dr. Mickelsen.

(3) It has been found that methionine may take the place of the extra casein needed when the high fat diet is fed.

(4) In confirmation of reports by others, obesity has been produced in mice injected with gold-thio glucose. Outwardly, this obesity appears similar to that produced by dietary means.

(5) Deficiencies of essential fatty acids, pyridoxine, pantothenic acid, and choline were produced, confirming reports of others. Vitamin A deficiency symptoms could not be produced on a vitamin A deficient diet in mice up to 8 weeks of age, even in the presence of large amounts of bentonite, known to increase the vitamin A requirement of the chick.

(6) No evidence could be found that mice required para-aminobenzoic acid or inositol, substances thought previously to be vitamins for the mouse.

(7) Using very young mice a deficiency of folic acid was produced in the absence of any sulfa drug, an antagonist, or other stress agent. This is contrary to the known results with rats and to most past results with mice.

Significance to NIAMD Research: The mouse has several important advantages for use in nutrition experiments inasmuch as they require little space and consume relatively small amounts of food. At the same time, they have a short life span, a fast growth rate, and rather exacting nutritional requirements. Thus, the project provides an important basic tool for the study of nutritional factors associated with various metabolic diseases such as arthritis, obesity, blood dyscrasias, and various nervous and nutritional disorders.

Proposed Course of Project: We anticipate putting emphasis on several phases of the obesity problem (factors necessary for maximum weight production, gold-thioglucose injections in combination with high fat diets, etc.), as well as making further studies on vitamin A metabolism (with Dr. Bieri), on folic acid deficiency, on the antibiotic growth promoting effect, and on the possible requirement for unidentified factors.

Analysis of NIH Program Activities

Budget Data and
Honors, Awards, and Publications Sheet

10. NIAMD-9
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. No cooperating units.

14. No parallel research in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis & Metabolic Diseases — INSTITUTE
2. Bioch mistry & Nutrition — LABOR.TORY OR BRANCH
3. Section on Biochemistry & Physiology of Nutrition — SECTION
4. _____ — LOCATION
5. NIAMD-10 — SERIAL NO.

6. Vitamin A and Carotene Metabolism
PROJECT TITLE

7. John G. Bieri
PRINCIPAL INVESTIGATOR(S)

8. Clifford J. Pollard
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: To obtain more information on the factors that affect vitamin A and carotene metabolism in the tissues of animals in order to aid in the elucidation of the mechanism whereby vitamin A metabolism is regulated in both normal and diseased individuals. Vitamin A tolerance studies have been performed on patients in collaboration with staff clinicians.

Methods Employed: Rats and chicks are depleted of their vitamin A reserves on synthetic type, vitamin A deficient diets. Various compounds are then administered, or nutrients withheld, to produce abnormal metabolism; e.g., as in diabetes or liver damage. Vitamin A, or carotene, is given to the animal and the fate of these substances followed in the tissues. The effect on growth is also noted.

Major Findings: (1) It was believed for many years that in human diabetics an impairment in carotene utilization, and subsequently in vitamin A metabolism, occurred. This relationship has been seriously questioned in recent years. However, a paper in 1954 claimed that diabetic rats had a decreased capacity for converting carotene to vitamin A. In the past year, studies in this laboratory with rats, have shown that, although the diabetic animal may under some conditions utilize carotene less efficiently than normal animals, the pancreas is not directly involved in the conversion of carotene to vitamin A. Also, the pancreas seems to be of only minor significance in the overall metabolism of vitamin A.

(2) It has been found in other experiments in this laboratory that in selenium poisoning in the rat a decreased utilization of both vitamin A and carotene occurs. The effect appears to be due to an increased destruction of vitamin A in the tissues. Vitamin E did not protect the vitamin A from this destruction.

Significance to NIAMD Research: These studies provide fundamental information about vitamin A which has importance in animal nutrition and in clinical medicine. Due to the unique instability of vitamin A, the assurance of an adequate intake is a problem in nutrition experiments. The destruction of vitamin A in various types of liver poisoning, the decreased utilization of carotene and the decrease in serum vitamin A which occurs in tuberculosis, prolonged fever, and in other diseases, indicate problems which must be considered clinically.

Proposed Course of Project: Effort will be directed toward elucidating the mechanism by which serum vitamin A levels are regulated. More evidence will be sought for the pathway by which carotene is converted to vitamin A in the body. The utilization of carotenoids other than carotene will be studied in the chick, rat, and mouse.

Analysis of NIH Program Activities

Budget Data and
Honors, Awards, and Publications Sheet

10. NIAMD-10
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. No cooperating units.

14. No parallel research in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases — INSTITUTE
2. Biochemistry and Nutrition — LABORATORY OR BRANCH
3. Section on Biochemistry and Physiology of Nutrition — SECTION
4. _____ — LOCATION
5. NIAMD-11 — SERIAL NO.
6. Effect of Ethylene Oxide on Foods — PROJECT TITLE
7. Dr. R. S. Yamamoto — PRINCIPAL INVESTIGATOR(s)
8. Dr. Olaf Mickelsen — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION:

Objectives: To determine the nutritional and biochemical changes resulting from the exposure of diets to ethylene oxide and related gases.

Methods Employed: Stock diet (Hunt Club Dog Food), purified diets, and various constituents of the diets are exposed to known concentrations of ethylene oxide for definite periods. The treated diets or diets prepared from the treated constituents are then fed to animals (rats, chicks). The growth of these animals is compared to the growth of control animals. Chemical and microbiological analyses are made of the diets to see which nutrients are destroyed. The effect of various conditions, such as pH and the moisture content of the diet during ethylene oxide treatment are also examined.

Major Findings: Exposure of the purified and stock diets to ethylene oxide causes a major nutritional alteration as shown by growth failure and, frequently, death of animals fed the treated diets. By analyses of the treated diets and by feeding diets prepared from these ingredients it has been shown that the primary damage is to the vitamins, especially thiamine. Other vitamins, such as riboflavin, niacin, and folic acid, are partially destroyed when exposed to ethylene oxide.

Studies show that choline compounds play an important role in bringing about this nutritional alteration. Very poor growth occurs when rats are fed a purified diet in which only the vitamin mixture containing choline chloride has been treated with ethylene

oxide. Omitting the choline during treatment of the vitamin mixture reduces the vitamin destruction to zero as shown by growth studies. The presence of choline chloride in the diet during ethylene oxide treatment caused reduced growth and death of rats in 4 to 5 weeks. The presence of choline citrate or choline bitartrate in place of the choline chloride during treatment produced a reduction in growth rate but no deaths. When the purified diet without choline was treated with ethylene oxide and the choline added later, growth and death were similar to that seen when the complete diet treated with the gas was fed. These findings suggest that some constituent in the diet can replace choline chloride in potentiating the destruction of vitamins.

Studies with the ethylene oxide treated stock diet showed that although the growth rate of rats fed the diet was markedly reduced, death did not occur. If the concentration of ethylene oxide routinely used were increased, death of rats did occur when they were fed the stock diet so treated. Only about half of the thiamine in the treated stock diet was destroyed by this treatment. Work of other investigators suggests that certain amino acids in the stock diet are inactivated by ethylene oxide treatment.

Significance to NIAMD Research: Ethylene oxide is being used on a small scale for the sterilization of a few food products. It is being used to a greater extent in the sterilization of a variety of pharmaceutical products, especially those which cannot be subjected to heat sterilization.

The nutritional changes produced by ethylene oxide treatment of the diets, especially the destruction of vitamins, is of importance in nutrition and toxicology. The results of these studies show that some substances used in the preservation or processing of foods may adversely affect the nutritional quality of the diet even though there is no trace of the original toxicant in the food after treatment. These findings emphasize the fact that foods should be tested from a nutritional as well as a toxicological standpoint. Testing the foods for the presence or absence of the original toxicant is not sufficient.

Proposed Course of Project: The mechanism involved in the ethylene oxide treatment of the vitamin in purified and stock diets will be studied and an attempt will be made to determine the conditions necessary for this destruction and the compounds formed as a result of ethylene oxide treatment.

Analysis of NIH Program Activities

Budget Data and
Honors, Awards, and Publications Sheet

10. NIAMD-11
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. No cooperating units.

14. No parallel research in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Hawk, E. A., and O. Mickelsen. Nutritional changes in diets exposed to ethylene oxide. Science 121: 442-444 (1955).

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases — INSTITUTE
2. Biochemistry and Nutrition — LABORATORY OR BRANCH
3. Section on Biochemistry and Physiology of Nutrition — SECTION
4. _____ — LOCATION
5. NIAMD-12 — SERIAL NO.

6. Experimental Obesity in Animals — PROJECT TITLE

7. Dr. O. Mickelsen — PRINCIPAL INVESTIGATOR(S)

8. Samuel M. Takahashi — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: To determine the biochemical and physiological alterations that occur in animals during the development of obesity and the subsequent loss of weight by obese animals.

Methods Employed: Rats are fed diets of varying fat content (20 to 63%). The growth rates and maximum weights attained on these diets are studied. Rats reaching a weight of approximately one kg. have been put on a variety of reducing regimens. These animals are maintained on the reducing diets to determine their longevity. Obese and normal weight rats have been exposed to simulated high altitudes and to high pressures to study the effects of these stresses on the physiological responses of the obese rat. An apparatus has been perfected which permits the determination of blood pressures in the intact, unanesthetized rat. Blood pressures are being taken of obese and control rats. Studies are being made of the possibility of recording heart rates in conjunction with the blood pressures.

Major Findings: Growth, maximum weight attained, and mortality at one year of age are essentially the same for the rats raised in individual suspended cages with screen bottoms and those raised two in a cage on sawdust. Some of the rats were raised on sawdust to see if the wire screens were responsible for the appearance of certain bone changes suggestive of osteoarthritis (work being done in cooperation with Dr. Leon Sokoloff). Concerning the different levels of dietary fat, at the end of one year it appears as though the highest percentage of obese rats are secured on the 20% level.

Continuation of the weight reduction studies indicated that the obese rats put on the stock diet or those fed the high fat diets in amounts equal to one half of that consumed prior to the initiation of the weight reduction regimen, or fed a high fat diet on an ad libitum basis two days each week, survived longer than those fed a high protein diet with either a low or moderate fat level. The rats on the restricted food intake (one half ration or fed two days per week) were very vicious when the feed cups were put into the cages; steel tongs had to be used for this purpose - at other times they could be handled without any trouble.

The preliminary study indicated that obese rats could not tolerate chronic exposure to simulated high altitudes as well as normal weight controls. However, further studies at a higher altitude showed no difference in the response of obese and normal weight rats as far as weight, blood changes, or mortality were concerned (work being done with Dr. Altland).

By incorporating an electronic filter into an amplifier system it has been possible to secure blood pressures from the tails of unanesthetized rats. The filter screens out all of the background static which made it impossible to use this technique prior to this improvement (work done with Mr. Frank Noble of the Heart Institute).

Significance to NIAMD Research: Obesity is listed as one of the major health problems today. The development of a means of producing obese animals entirely by dietary procedures will permit the study of obesity in animals uncomplicated by other physiological changes. The primary emphasis of this work will be on the effects of a reduction in body weight in obese animals.

Proposed Course of Project: Obese rats will be carried on the reducing regimens until they have attained the weight of normal adults. They will then be studied to see if their physiological and metabolic functions are the same as those in animals that were never obese. These studies will include blood pressure measurements, body composition, reproductive capacity, incidence of disease, longevity, etc. Studies will be made to see whether there are any differences between obese and normal weight rats when rapidly decompressed from high atmospheric pressures (work being done with Dr. Altland and Dr. Webb Haymaker (Armed Forces Institute of Pathology)).

Analysis of NIH Program Activities

Budget Data and
Honors, Awards, and Publications Sheet

10. NIAMD-12
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

Laboratory of Physical Biology, NIAMD
Laboratory of Pathology and Histochemistry, NIAMD
Laboratory of Technical Development, NHI
Armed Forces Institute of Pathology, Walter Reed Hospital

14. No parallel research in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Bragdon, J. H., and O. Mickelsen. Experimental atherosclerosis in the rat. Am. J. Path. 31: 965, 1955.

Mickelsen, O. Nutrition and Alcoholism--A Review. J. Am. Diet. Assoc. 31: 570, 1955.

Caster, W. O., and O. Mickelsen. Serum Vitamin A Level: A Critique of Methods and Significance. Am. J. Clin. Nutr. 3; 409, 1955.

Hundley, J. M., O. Mickelsen, N. Mantel, R. N. Weaver, and R. C. Taber. Height and weight of first-grade children as a potential index of nutritional status. Am. J. Pub. Health 45: 1454, 1955.

Mickelsen, O., S. Takahashi, and C. Craig. Experimental Obesity. I. Production of obesity in rats by feeding high fat diets. J. Nutr. (in press).

Keys, A., J. J. Anderson, and O. Mickelsen. Serum cholesterol in men in basal and non-basal states. Science (in press).

Mickelsen O. Intestinal synthesis of vitamins in the non-ruminant. Vits. and Hormones (in press).

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Laboratory of Biochemistry and Nutrition
LABORATORY OR BRANCH

3. Biochemistry and Physiology of Nutrition
SECTION

4. _____
LOCATION

5. NIAMD-13
SERIAL NO.

6. Blood Glucose Regulation in Factor 3 Deficiency and in Dietary Necrotic Liver Degeneration
PROJECT TITLE

7. W. Mertz (Guest)
PRINCIPAL INVESTIGATOR(S)

8. K. Schwarz
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: (a) To elucidate the disturbances of glucose metabolism during the latent phase of dietary necrotic liver degeneration, (b) to investigate the influence of various dietary factors on the impairment of intravenous glucose tolerance, and (c) to study the mechanism through which the liver regulates the uptake of glucose by tissues.

Methods Employed: In rats on rations producing dietary necrotic liver degeneration, fasting blood glucose levels and intravenous glucose tolerance were measured under varying conditions. The prophylactic effects of the feeding of Factor 3, vitamin E, cystine, and other substances on glucose tolerance were determined in groups of animals at various stages of the disease. The curative action of Factor 3 given by stomach tube or by injection was tested and standardized for the assay of Factor 3. Homogenates and extracts from normal and deficient livers and other organs were tested as to their influence on the impaired intravenous glucose tolerance and on the glucose uptake of eviscerated animals.

NIAMD-13
SERIAL NO.

Major Findings: During the latent phase, several weeks before anatomical lesions are visible in the liver, glucose removal is seriously impaired. Vitamin E has no effect on the impairment, while cystine has a delaying action on its development. Factor 3 preparations protect fully against it.

The impaired tolerance is cured by stomach tubing or by injection of Factor 3 concentrates. Fourteen to 18 hours after such injections, glucose removal is normal. Tests performed with a number of different Factor 3 preparations agree with the routine prophylactic Factor 3 assay.

Rats showing the impairment of glucose tolerance are suitable for investigating the influence of the liver on peripheral glucose uptake. Homogenates or extracts from normal liver, injected simultaneously with the glucose, bring the impaired tolerance immediately back to normal (glucose-removal factor). The glucose-removal factor enhances the impaired glucose uptake in eviscerated rats.

Significance to NIAMD Research: The results show that Factor 3 is a food factor of its own. They have made it possible to assay for Factor 3 independently from vitamin E. The project opens a new approach to the understanding of the mechanism of blood glucose regulation and its disturbances. The latter play a role in many diseases, for example, in diabetes.

The Proposed Course of the Project: Further Factor 3 tests with the intravenous glucose-tolerance method are in progress. The prophylactic test is used for crude dietary materials which contain vitamin E in forms which cannot be removed by extraction; the curative assay is used for fractions of which only very small quantities are available. Experiments to clarify the relation between Factor 3 and the glucose-removal factor have been initiated. It is planned to investigate the distribution of the glucose-removal factor in various organs, its physical and chemical properties, and the mechanism by which it exerts its action.

Analysis of NIH Program Activities

Budget Data - and
Honors, Awards, and Publications Sheet

10. NIAMD-13
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S)(ITEM 10)

Brewer's Yeast Council, Inc. (Research Fellowship)

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES, OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

No parallel research in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Mertz, W., and Schwarz, K., Impaired Intravenous Glucose Tolerance as an Early Sign of Dietary Necrotic Liver Degeneration, Arch. Biochem. & Biophys. 58, 504 (1955).

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Laboratory of Biochemistry and Nutrition
LABORATORY OR BRANCH

3. Biochemistry and Physiology of Nutrition
SECTION

4. _____
LOCATION

5. NIAMD-14
SERIAL NO.

6. Isolation and General Significance of Factor 3 Against Dietary Necrotic Liver Degeneration
PROJECT TITLE

7. K. Schwarz
PRINCIPAL INVESTIGATOR(S)

8. L. H. Mason and W. Mertz (Guest)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To isolate and chemically identify Factor 3 and to clarify its biochemical and medical significance in general.

Methods Employed: Conventional isolation and fractionation procedures are used, such as chromatography, precipitations, solvent extractions, etc., and new techniques are developed for the unidentified factor. The activity of purified fractions is assayed in a prophylactic dietary test, also by the intravenous glucose-tolerance method (see Mertz project), as well as with the Warburg technique (respiratory decline).

Major Findings: A new method (Dowex process) for large-scale preparation of Factor 3 concentrates has been developed and subsequent steps with this material have been worked out. Small amounts of highly purified, microcrystalline fractions have been obtained after 10-14 steps of fractionation. From these, several compounds have been isolated in pure form, but sufficient quantities for accurate prophylactic assays have not yet been available.

Major Findings (Continued):

Tests for Factor 3 activity, using the prophylactic assay, have been performed on a total of 70 different natural-source materials. Factor 3 is primarily found in materials of animal origin; plant products are usually poor in activity. Tests with the glucose-tolerance method, which is independent from vitamin E, showed that some commercial stock diets for rats and other animals are deficient in Factor 3.

Liver-necrosis producing diets were fed to mice, in collaboration with W. DeWitt (NMI). In this species, not only the liver, but also the heart, peripheral muscle, kidneys, and probably the pancreas, developed necrotic degeneration. Severe necrosis of the heart muscle precedes liver necrosis by several weeks, with an incidence of almost 100%. Factor 3 concentrates prevented these lesions.

Factor 3 preparations were assayed against exudative diathesis (G. M. Briggs). The lesion is produced by a modification of our Torula yeast diet. This fatal deficiency was prevented by crude, as well as purified Factor 3 preparations.

Vitamin E, which prevents dietary necrotic liver degeneration, is well defined as an antioxidant. Therefore, a number of commercial, synthetic antioxidants were tested for activity in preventing liver necrosis. Three out of 14 different substances were highly effective. One of these, DPPD (N,N'-diphenyl-phenylenediamine), was almost as active as vitamin E.

The same series of antioxidants was tested for the reversion of respiratory decline after intraportal injection. Those antioxidants which were effective when added to the diet also reverted respiratory decline of liver tissue in the intraportal test. DPPD was 2.4 times as active as vitamin E. The various antioxidants were also effective in preventing respiratory decline of liver slices when added directly to the Warburg medium. When added in this way, vitamin E was inactive. DPPD protected in very small doses (.2 to .4 μg).

Significance to NIAMD Research: Factor 3 has the characteristics of a new vitamin, is active in preventing marked and fatal anatomical lesions in several species, has a demonstrated role in maintaining normal tissue respiration, and in glucose utilization. Therefore, it can be anticipated to have an important role in biochemical processes and be of significance in human disease.

Proposed Course of the Project: The continuation of the fractionation and isolation studies as outlined above, in order to obtain Factor 3 in pure form, to identify its chemical characteristics, to elucidate its role in metabolism, and to make it available for clinical research.

Analysis of NIH Program Activities

Budget Data - and
Honors, Awards, and Publications Sheet

10. NIAMD-14
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

Section on Helminthic Diseases, Laboratory of Tropical Diseases, NMI

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES, OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

No parallel research in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Schwarz, K., The Role of Yeast in the Production and the Prevention of Dietary Liver Necrosis; Factor 3 in Brewer's Yeast, Modern Brewery Age 52, No. 6, 44 (1954), and 53, No. 1, 72 (1955).

2. Schwarz, K., Chernick, S. S., Rodnan, G. P., Moe, J. G., and Mertz, W., Dietary Necrotic Liver Degeneration: Occurrence of a Specific Metabolic Defect Reversible by Intraportal Vitamin E, 3rd International Congress on Vitamin E, Venice, Italy, (1955), in press.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Fellow, New York Academy of Sciences (K. Schwarz)

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Laboratory of Biochemistry and Nutrition
LABORATORY OR BRANCH

3. Biochemistry and Physiology of Nutrition
SECTION

4. _____
LOCATION

5. NIAMD-15
SERIAL NO.

6. Metabolic Changes in the Development of Dietary Liver Necrosis
PROJECT TITLE

7. S. S. Chernick
PRINCIPAL INVESTIGATOR(S)

8. K. Schwarz and M. G. Piccardo (Guest)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine the chain of events which leads from nutritional deficiency, through metabolic changes, to the final stage of dietary necrotic liver degeneration.

Methods Employed: Tissue metabolism studies (Warburg technique) were performed on slices, homogenates, and other preparations from livers and other organs at various stages of the deficiency. The utilization of isotopically labelled acetate was investigated. Coenzyme A levels were measured in livers of animals on various diets. The effects of the intraportal injection of emulsified tocopherols on respiratory decline were determined. The action of 2,4-dinitrophenol on respiratory decline was investigated, and cellular elements were fractionated (method of Hogeboom and Schneider) for the study of oxidative phosphorylation in mitochondria.

Major Findings: A characteristic metabolic lesion (respiratory decline) in slices of livers from rats fed the necrogenic diet, during the latent phase of the disease, has been discovered and described in detail (1). The utilization of acetate-2-C^{14} for fatty acid synthesis, ketone body production, and CO_2 formation breaks down in respiratory decline (2). Coenzyme A levels are diminished in livers of animals on necrosis-producing diets; this is not related to respiratory

decline or to death from liver necrosis (3). Respiratory decline is reverted within minutes by the injection of emulsified vitamin E into the portal vein. The 4 different forms of vitamin E (α-, β-, γ-, and δ-tocopherol) differ widely in activity when tested with this method (4). The γ-form was almost as active as α-tocopherol. The stimulatory and the inhibitory effects of varying concentrations of 2,4-dinitrophenol were masked in prenecrotic liver slices showing respiratory decline. The site of the respiratory lesion, which appears to reside in oxidative phosphorylation, is separate from that of the action of dinitrophenol.

Mitochondrial preparations from normal-appearing livers, during the latent phase of dietary necrotic degeneration, have very low rates of oxygen and phosphorus uptake (P/O ratios). The defect is repaired largely by the addition of DPN. DPN measurements in deficient mitochondria (N. Kaplan, Johns Hopkins University, Baltimore) showed levels far below normal.

Significance to NIAMD Research: The mechanisms by which vitamin deficiencies produce functional and anatomical damage and death are not well known. Clarification of the sequence of events leading to liver necrosis as the end result of a nutritional deficiency is expected to lead to basically important knowledge and to a better understanding of these diseases. The biochemical aspects of necrosis, i.e., death of areas of tissue, are until now poorly understood, even though necrosis occurs in many different diseases. It is hoped that a logical basis can be developed for new ways of preventing necrotic lesions.

Proposed Course of Project: Detailed enzymatic studies with mitochondria and other cell particles are planned in order to identify the site of the primary defect. Formation and breakdown of DPN, ATP, and also of ribonucleic acid, are scheduled to be investigated with labelled substrates. The intracellular distribution of vitamin E will be studied with C^{14}-labelled α-tocopherol.

Electron microscope investigations of liver cells during the development of the disease are under way. These are coordinated with metabolic and microscopic investigations. The investigations will be extended to various organs of the mouse, in which not only the liver, but also the heart, musculature, and kidneys undergo dietary necrotic degeneration.

Analysis of NIH Program Activities

Budget Data - and
Honors, Awards, and Publications Sheet

10. NIAMD-15
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S)(ITEM 10)

Section on Molecular Biophysics, Laboratory of Physical Biology, NIAMD

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

No parallel research in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Chernick, S. S., Moe, J. G., Rodnan, G. P., and Schwarz, K., A Metabolic Lesion in Dietary Necrotic Liver Degeneration, J. Biol. Chem. 217, 829 (1955).

2. Rosecan, M., Rodnan, G. P., Chernick, S. S., and Schwarz, K., Acetate-2-C^{14} Utilization in Dietary Necrotic Liver Degeneration, J. Biol. Chem. 217, 967 (1955).

3. Chernick, S. S., Moe, J. G., and Schwarz, K., Dietary Necrotic Liver Degeneration and Coenzyme A, Proc. Soc. Exp. Biol. and Med. 89, 499 (1955).

4. Rodnan, G. P., Chernick, S. S., and Schwarz, K., Reversal of the Respiratory Lesion in Dietary Necrotic Liver Degeneration by Intraportal Injection of Various Tocopherols, J. Biol. Chem., (1956), in press.

5. Chernick, S. S., and Moe, J. G., Effects of Hypophysectomy on the Coenzyme A Content of Rat Liver, Endocrinology, (1955), in press.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY

3. Endocrinology
SECTION

4. _____
LOCATION

5. NIAMD-16
SERIAL NUMBER

6. The influence of the Central Nervous System on cardiovascular-renal function
PROJECT TITLE

7. Dr. Edward W. Hawthorne and Dr. Joseph Johnson (Howard University) Dr. Evelyn Anderson and Dr. Kathryn Knowlton
PRINCIPAL INVESTIGATORS

8. Mr. E. M. Mapp (Howard University), Dr. David McK. Rioch (Walter Reed), Dr. William T. Spence (Georgetown University).
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To study the cardiovascular-renal functional alterations incident to midbrain and spinal cord transection with the following major views in mind:

(1) To gain insight into the acute and chronic effects of loss of discreet neural influences (cortical, hypothalamic, and spinal) on cardiac and renal function.

(2) To understand the relationship between renal electrolyte and water excretion and the general fluid and electrolyte balance in dogs following midbrain and spinal cord transections.

Methods Employed: Dogs are used and observations made during a control, operative, and post-operative period. The dogs are maintained on a rigidly controlled diet and daily "intake-output" records are kept. Throughout each period determinations are made of arterial pressure, renal clearances, cardiac output, measurements of various fluid "spaces" and balance studies with respect to nitrogen, sodium and water, and creatinine excretion. We are attempting to develop a method for the bioassay

of aldosterone content in dog urine, using the rat. It is felt that the animal will be highly sensitive to microquantities of aldosterone.

Major findings: It appears that acutely following midbrain transection there occurs a marked reduction in effective glomerular filtration, effective renal plasma flow, and cardiac output. The reductions observed in the several parameters ranged from 30-50% of normal. Associated with these findings there was observed a marked elevation in calculated total peripheral and renal resistance. The implication is that acutely following midbrain transection in the dog there occurs a marked arteriolar spasm. This latter may, by increasing the left ventricular work load, lead to the changes in cardiac output, effective renal plasma flow, and glomerular filtration rate.

Significance to arthritis and metabolic research: The prolonged survival of dogs with midbrain and cervical cord transections affords one an unusual opportunity to study the role of the central nervous system in the regulation of renal and cardiac function. Exactly what part the nervous system plays in the regulation of normal fluid balance, cardiac output, arterial pressure and general renal function is not known at the present time. These studies bear directly on our desire to understand clearly the mechanism of pathophysiologic alterations in the above parameters occurring in patients with specific nervous, endocrine, cardiac output, arterial pressure, extracellular fluid space and blood volume, along with balance studies including nitrogen, fluid and electrolyte balances. Following development of a suitable bioassay, we intend to observe the changes in urinary aldosterone excretion in these dogs.

Proposed course of project: It is intended to study a series of dogs with midbrain and spinal cord transection, the acute and chronic alterations in renal and cardiac function, along with changes in the fluid and electrolyte balance. We plan to continue studies of the changes in glomerular filtration, renal plasma flow, cardiac output, arterial pressure, extracellular fluid space and blood volume, along with balance studies including nitrogen, fluid and electrolyte. Following development of a suitable bioassay, we intend to observe the changes in urinary aldosterone excretion in the dogs.

Budget Data
and
Honors, Awards, and Publications Sheet

10. NIAMD-16
SERIAL NUMBER

12.
BUDGET ACTIVITY: Research

13.
IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 OR 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S).

AMSGS, Army Medical Center.
Howard University School of Medicine.
Georgetown University School of Medicine.

14. No parallel research.

16.
LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Alterations of adrenal cortical and ovarian activity following hypothalamic lesions. Endocrinology 57, No. 1 pp. 44-54, July, 1955. Gert L. Laqueur, S. M. McCann Leon H. Schreiner, Eugenia Rosemberg, David McK. Rioch and Evelyn Anderson.

Chapter 21, Volume 2, "Clinical Neurology", A. B. Baker, Editor, entitled "Disorders of the Hypothalamus and Pituitary Gland" by Webb Haymaker and Evelyn Anderson. Harper and Brothers, 1955.

17. No honors and awards.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY

3. Endocrinology
SECTION

4. _____
LOCATION

5. NIAMD-17
SERIAL NUMBER

6. Analysis of urinary steroids in mice bearing a transplanted ACTH producing tumor
PROJECT TITLE

7. Dr. Hildegard Wilson (Guest) and Dr. Robert Bahn
PRINCIPAL INVESTIGATORS

8. Dr. Jacob Furth (Harvard University)
OTHER INVESTIGATOR

9 PROJECT DESCRIPTION

Objectives: Little or nothing is known about the steroids in mouse urine, in spite of the widespread use of this animal in endocrinological experiments. Although the urine is not the major excretory pathway for steroids in mice, a larger proportion is so excreted than in rats. With the expectation that the output would be elevated because of the tumor, the undertaking was deemed profitable. Characterization of the types of steroid being excreted would indicate what steroid hormones were actually responsible for the endocrine changes observed.

Correlation of the physiological effects of the ACTH tumor with steroid secretion in the tumor bearing mice, as judged by urinary steroid metabolic end products.

Methods employed: All the urine excreted by 3 tumor bearing mice was collected for 2 months. To effect hydrolysis of the various steroid conjugates, it was first incubated with -glucuronidase, then acidified to pH 0.8 and continuously extracted with ether, and finally heated with 5% H_2SO_4 and again extracted. The neutral extract fraction was chromatographed first on a partition column which separated corticosteroids from ketosteroids. These two fractions were then again chromatographed on paper by the Zaffaroni technique, and rechromatographed by the Bush technique. All regions were eluted, assayed by appropriate micro procedures, and further characterized where possible.

Major findings: Several highly polar corticosteroids, (like cortisone, cortisol, or tetrahydro cortisone) were present in minute amounts totalling about 0.2 ug. per mouse per day. A band containing about 0.05 ug. moving like aldosterone, was negative to a bioassay. The dominant corticoid appeared opposite the corticosterone reference standard, and gave spot tests consistent with the presence of 0.3 ug. per day of this steroid. Total corticoids were about 0.6 ug. per day. The most abundant class of steroid metabolites (about 20 ug. per day) were $C_{19}O_3$ 17-ketosteroids retaining the 11-oxygen function, and therefore probably of adrenal origin. $C_{19}O_2$ 17-ketosteroids, which include testicular metabolites, totalled about 10 ug. per day.

Significance to arthritis and metabolic research: There are several important applications of these studies. Since some of the tumors arose in mice exposed to ionizing radiation and may have their counterpart in the human the data accumulated may eventually have clinical significance. Beyond this direct clinical application the tumors and tumor bearing mice provide experimental models from which information is being gained concerning (1) the relationship of the adrenal gland to water metabolism and reproduction and (2) biosynthesis of steroid hormones by the mouse adrenal.

Proposed course of project: Procedures directed toward identification of several of these metabolites are now underway. It is of importance to be certain that corticosterone was the major corticosteroid in the tumor mouse urine, as this would indicate its secretion by the adrenal cortex, and that it may have been responsible for the observed salt retention. Since some 11-oxy-17-ketosteroids are known to be androgenic, it would also be valuable to definitely establish the presence of such a compound.

Budget Data
and
Honors, Awards, and Publications Sheet

10. NIAMD-17
SERIAL NUMBER

12.
BUDGET ACTIVITY: Research

Harvard University School of Medicine.
13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S).

14. No parallel research

16. None
LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

17. No honors or awards.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY

3. Endocrinology
SECTION

4. _____
LOCATION

5. NIAMD-18
SERIAL NUMBER

6. The influence of the central nervous system on endocrine activity
PROJECT TITLE

7. Dr. Hildegard Wilson (Georgetown)
PRINCIPAL INVESTIGATOR

8. Dr. David McK. Rioch (Walter Reed), Dr. William T. Spence (Georgetown), Mr. John Borris (Walter Reed), and Mrs. Mary Garrison (Georgetown).
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The influence of the central nervous system on ACTH-adrenal cortical activity, as based on transections of the midbrain and spinal cord.

To apply methods which have been developed during the past year for the analysis of steroids in the urine and plasma of dogs. To study by these means, the variations in adrenocortical function before and after transections of the midbrain and spinal cord.

Methods employed: A method for the analysis of urinary steroids in dogs has been developed. The steroid conjugates are hydrolyzed first with -glucuronidase (which splits glucuronides) and then by acidification to pH 0.8 followed by continuous ether extraction (which splits sulfates) The neutral fraction is prepared by washing the total extract with 1N NaOH. This fraction is chromatographed on a partition column using aluminum silicate as the supporting phase and 50% ethanol as the stationary phase. Elution with hexane yields the $C_{19}O_2$ 17-ketosteroids, and hexane plus increasing amounts of chloroform gives first $C_{19}O_3$ steroids and then corticosteroids. Each of these fractions is assayed by the chromic acid-ketosteroid procedure, which determines 17-ketosteroids and 17-hydroxy corticosteroids. The corticosteroid containing fraction is also assayed for 21-hydroxy steroids by measuring the formaldehyde formed after oxidation with periodic acid.

NIAMD-18
SERIAL NO.

Major findings: It has been found that intramuscular injection of ACTH (120 mg. per day) to a dog gave increases of 2 to 10 fold in various classes of urinary steroids Thus, the procedure was adequate to detect alterations in adrenocortical function. Transection of the spinal cord was followed immediately by a 2-3 fold increase in urinary steroids lasting for 4 days after the operation The stress of a laparotomy without anaesthetic in this spinal dog also caused a 2-3 fold increase in steroid excretion. The cord transection therefore does not seem to have blocked the release of pituitary ACTH in response to stress. On the other hand, transection of the midbrain in another dog produced a negligible increase in urinary steroids, and a laparotomy 4 weeks later also caused little or no rise. Activation of pituitary ACTH hence appears to have been blocked by the midbrain transection.

Significance to arthritis and metabolic research: The method for the analysis of steroids in dog urine is the first successful procedure developed for this purpose. It opens up opportunities for following changes in either adrenocortical or gonadal function under circumstances which can be studied in dogs better than in humans Moreover, the method can easily be adapted to the analysis of human urine. Analysis of urine has an advantage over plasma analysis for steroids in that its procurement does not constitute an operative procedure, so that accurate evaluation of existing adrenocortical activity is possible. The results so far obtained throw new light on the involvement of the central nervous system in the pituitary response to stress.

Proposed course of the project: The same type of study will be continued in greater detail. The dogs will be subjected to the same stress procedures before and after the central nervous system lesions are placed, and the quantitative responses will be compared. The stress procedures will be repeated at intervals to see whether other neural or humoral pathways become established.

Parallel metabolic studies will be done throughout so that adrenal cortical activity can be definitely correlated with alterations in the excretion of nitrogen, creatine, and electrolytes (in cooperation with Dr. Knowlton) and thyroid activity (in cooperation with Dr. Bates). It is hoped to carry out analyses for aldosterone excretion in these dogs The analyses for peripheral blood and adrenal blood steroids planned last year will also be performed if this is feasible.

Budget Data
and
Honors, Awards, and Publications Sheet

10. NIAMD-18
SERIAL NUMBER

12.
BUDGET ACTIVITY - Research

13.
IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 OR 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S).

Walter Reed
Georgetown University

14. No parallel project.

16.
LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. A rapid micro method for the determination of 17-hydroxy- and 17-ketosteroids. Hildegard Wilson and Richard Fairbanks Arch. Biochem. & Biophys. 54, 440 (Feb. 1955).

2. A micromethod for the detection and assay of steroid C_{21} 17-hydroxy- -glycols. Hildegard Wilson and Richard Fairbanks Arch. Biochem. & Biophys. 54, 457 (Feb. 1955).

3. Studies on the metabolism of adrenal cortical steroids in the synovial cavity in rheumatoid arthritis. Hildegard Wilson, Richard Fairbanks, Currier McEwen and Morris Ziff. Annals of the New York Academy of Sciences 61, 502 (1955).

4. Metabolites of hydrocortisone and cortisone in synovial fluid in rheumatoid arthritis. Hildegard Wilson, Richard Fairbanks, Dominick Scialabba, Currier McEwen and Morris Ziff. Jour. Clinical Endocrinol. & Metabolism. Accepted, February, 1955. To appear January, 1956.

17 No honors and awards.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY

3. Endocrinology
SECTION

4. _____
LOCATION

5. NIAMD-19
SERIAL NUMBER

6. Prolactin in human blood and urine
PROJECT TITLE

7. Dr. Robert W. Bates and Dr. Robert C. Bahn
PRINCIPAL INVESTIGATORS

8. Mr. Tulane B. Howard
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION

Objectives: To settle the question of determining the presence or absence of prolactin in human urine and blood.

Methods employed: Prolactin is assayed using the cropsacs from pigeons. Injections are made intradermally over the crop or intramuscularly in the pigeon. Blood serum, urine concentrates, and prolactin from human pituitaries were studied using various histochemical techniques.

Major findings: The mucosa of cropsacs from pigeons injected, either systemically or locally, with prolactin showed gross and microscopic proliferation, large increase in size of intracellular fat droplets and an intense cytoplasmic basophilia. The latter indicates an increase in cytoplasmic ribonucleic acid concentrations which has been correlated with protein synthesis.

In contrast, when injected systemically, urine and blood fractions never stimulated the cropsac. Local intradermal injection of such fractions or of many non-specific substances, such as ethanol or tannic acid, will cause gross and histological proliferative changes in the cropsac mucosa locally and usually an inflammatory reaction submucosally, but the intense cytoplasmic basophilia and increase

in lipids are absent The conclusion is that no prolactin or such is detectable in human blood or urine

Significance to arthritis and metabolic research. An endocrine explanation for gynecomastia and persistent lactation in patients has long been sought On the basis of nonspecific changes in the cropsac, certain investigators have reported finding prolactin in the urine in such patients. This explanation will have to be reconsidered in the light of the above findings of no detectable amount of prolactin in blood and urine

Proposed course of project: Completed

Budget Data
and
Honors, Awards, and Publications Sheet

10. NIAMD-19
SERIAL NUMBER

12.
BUDGET ACTIVITY - Research

13. No cooperating unit.

14. No parallel research

16.
LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Diabetogenic Action of Prolactin. B. A. Houssay, E. Anderson, R. W. Bates and Choh Hao Li. Endocrinology, 57, No. 1, July, 1955, pp. 55-63.

17. No honors and awards.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY

3. Endocrinology
SECTION

4. _____
LOCATION

5. NIAMD-20
SERIAL NUMBER

6. Study of a mouse pituitary tumor which elaborates ACTH and melanophore stimulating hormone (MSH)
PROJECT TITLE

7. Dr. Robert Bahn and Dr. Peter G. Condliffe
PRINCIPAL INVESTIGATORS

8. Dr. Jacob Furth (Harvard University) and Dr. Raul Echenique (Havana, Cuba).
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: A transplantable pituitary tumor was originally found in mice by Dr. Furth, which stimulate the adrenals of the host. The purpose of these studies is to establish the probable chemical nature of the adrenal stimulating hormone present in the tumor, by comparing it with standard ACTH and also studying the MSH and comparing ft with MSH of pituitary glands of other species.

Methods employed: Extracts are made of beef pituitary glands and mouse pituitary tumors. These are fractionated and studied by countercurrent distribution, electrophoresis and chromatography. The distribution of hormone in the various fractions is estimated by bioassay, i. e., ascorbic acid depletion for ACTH and isolated frog skin for MSH.

Major findings: About one hundred grams of tumor powder have been accumulated and this is being used as a source of hormone. It has been found that in addition to being able to stimulate the adrenals, the tumor powder contains MSH. One question to be answered is whether the tumor contains both ACTH and MSH as separate hormones, or whether the MSH activity is to be attributed to the ACTH content, since it is known that some preparations of ACTH possess inherent MSH activity which can be enhanced by treatment with alkali. Treatment of tumor extracts with alkali enhances their MSH potency, suggesting that the hormone present is more like ACTH in this respect. To make further comparisons, MSH has been prepared from beef posterior pituitary gland and the same procedure is being employed with tumor powder.

Significance to arthritis and metabolic research: This study should throw light on the question of the chemical relationship between ACTH and MSH.

Proposed course of project: The actual amount by weight of corticotropin-like substance is too small for isolation from the tumor to be practicable. However, due to the sensitivity of the available bioassay, it is possible to follow up the hormonal activity through a number of chemical procedures. By subjecting tumor extracts, purified ACTH and MSH, to identical procedures, it should be possible to ascertain which of these two hormones most closely resembles the adrenal stimulating hormone present in the tumor.

Budget Data
and
Honors, Awards, and Publications Sheet

10. NIAMD-20
SERIAL NUMBER

12. BUDGET ACTIVITY: Research

13. No cooperating units.

14. No parallel research.

16. No publications.

17. No honors and awards.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Biochemistry and Nutrition 3 Endocrinology
LABORATORY

4. _____
LOCATION

5. NIAMD-21
SERIAL NUMBER

6. Insulin Studies
PROJECT TITLE

7. Dr. Evelyn Anderson and Dr. Robert W. Bates
PRINCIPAL INVESTIGATORS

8. Mrs. Frances W. Franco and Mr. Jerome Cornfield (Biometrics)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objective of this project is to study mechanisms which control insulin release in a variety of endocrine states.

Methods employed: A method for the bioassay of insulin which has just been completed is used to measure insulin content in extracts of pancreas and blood. An attempt will be made to apply this method to the direct measurement of insulin in untreated blood. A technique for the perfusion of the pancreas in situ has been developed. This is being used at present to prepare isotopic labeled insulin

Major findings: In the method for insulin assay a reproducible dose-response curve has been attained which is capable of detecting as little as 250 microunits of insulin. Preliminary attempts at isolation of crystalline insulin from the pancreas of individual dogs has been partially successful.

Significance to arthritis and metabolic research: A satisfactory method for the assay of insulin in blood would prove a useful tool in the study of problems in carbohydrate metabolism and in diabetes.

Proposed course of project: 1. To prepare isotopically labeled insulin. 2. To explore the problem of measuring insulin levels in various clinical and experimental conditions. 3. To study factors relating to insulin content of pancreas and factors influencing release of insulin.

Budget Data
and
Honors, Awards, and Publications Sheet

10. NIAMD-21
SERIAL NUMBER

12. _____
Budget Activity - Research

13. Office of Biometry - NIH
IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 OR 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S).

14. No parallel research.

16. No publications.

17 No honors and awards.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY

3. Endocrinology
SECTION

4. _____
LOCATION

5. NIAMD-22
SERIAL NUMBER

6. Isolation, chemistry and bioassay of anterior pituitary hormones with particular reference to thyroid stimulating hormone (TSH)
PROJECT TITLE

7. Dr. Robert W. Bates and Dr. Peter Condliffe
PRINCIPAL INVESTIGATORS

8. Mr. Tulane Howard, Miss Alice Laskey and Dr. Jacob Furth (Harvard University)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To isolate the anterior pituitary hormones (thyrotrophin, ACTH, growth hormone, gonadotrophins, and the prolactin) and to determine their chemical composition. To compare/purified hormones isolated from pituitary glands with those isolated from transplantable mouse pituitary tumors.

Methods employed: Extracts are made of pituitary glands and of mouse pituitary tumors. These extracts are fractionated and studied by salt fractionation, solvent fractionation, isoelectric precipitation, preparative electrophoresis, chromatography and countercurrent distribution. Identification and quantitative estimation of the amino acid constitution of purified fractions are made by paper chromatography after hydrolysis with or without formation of suitable derivatives. The potency of all fractions must be determined by bioassay using iodine depletion in the chick thyroid for thyrotrophin; bioassay using ascorbic acid depletion of the adrenal of the rat for adrenocorticotrophin; cropsac weight increase in pigeons for prolactin; seminal vesicle weight increase in rat for luteinizing hormone; uterine and ovarian weight increase in rat for follicle stimulating hormone and body weight increase in the rat for growth hormone.

Major findings: Isolation of thyroid stimulating hormone (TSH). A TSH preparation has been extracted from bovine pituitary powder which is 200 times more potent per mg. than the starting powder. Extensive studies have been made on methods with the ion exchange columns of modified cellulose used for isolating TSH. With proper methods the columns make possible a 5-10 fold purification of TSH with little loss in total potency. Preliminary amino acid analysis of the most potent TSH fractions indicates lack of methionine and possibly cystine as well as low tyrosine and tryptophane content. Electrophoretic studies indicate an isoelectric point between pH 7 and 9.

The bioassay method for TSH which has been developed in this laboratory can detect approximately 2 milliunits (0.2 micrograms) of TSH. The precision of the assay is 0.12-0.15, which is unusually good for a bioassay. TSH has been detected in 0.002 ml. of the blood plasma of radiothyroidectomized mice bearing transplantable pituitary tumors that produce TSH. This is approximately 1 unit/ml. plasma. The concentration of TSH in human plasma is less than 0.001 unit/ml.

We have been cooperating with a subcommittee of the Endocrine Study Section (NIH). Bioassays for TSH and prolactin have been made on commercial samples of pituitary hormones.

Significance to arthritis and metabolic research: The mechanism of action of pituitary hormones is not, as yet, understood. Progress will depend upon availability of pure hormones and the accuracy and simplicity of the bioassay procedures. This progress has been advanced by our improved methods for preparation and bioassay. It is hoped that these methods will permit determination of blood levels of TSH in patients.

Proposed course of project: Efforts to prepare pure TSH will be continued and, when successful, full chemical and physical characterization will follow. Studies on the effect of TSH on thyroid physiology in the chick will continue. Time course studies will be made on the blood levels of TSH in radiothyroidectomized mice implanted with TSH producing tumors to determine when the blood levels of TSH rise. Fractionation studies on mouse blood may lead to methods for isolating TSH from blood of man where the concentration is low.

Budget Data
and
Honors, Awards, and Publications Sheet

10. NIAMD-22
SERIAL NUMBER

12.
BUDGET ACTIVITY - Research

13. Harvard University School of Medicine
IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S).

14. No parallel research.

16. No publications.

17. No honors and awards.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY

3. Endocrinology
SECTION

3. _____
LOCATION

5. NIAMD-23
SERIAL NUMBER

6. The influence of the central nervous system on creatine and creatinine metabolism as based on transections of midbrain and spinal cord in dogs
PROJECT TITLE

7. Dr. Kathryn Knowlton
PRINCIPAL INVESTIGATOR

8. Mr. Leonard H. Kedda, Dr. W. T. Spence (Georgetown) and Dr. David Rioch (Walter Reed)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To study the role of the central nervous system in creatine and creatinine metabolism and the mechanisms by which its effects are produced.

Methods employed: Transections of the midbrain and spinal cord in the dog are made with careful neurosurgical technique. The animals are maintained on a food intake kept constant throughout the study. Urinary creatine and creatinine are determined for these animals. Creatine labeled with N^{15} is administered, and the distribution of this isotope in the urine followed. For this purpose, creatine and creatinine are isolated, purified, and prepared for determination of their N^{15} abundance by the mass spectrometer.

Major findings: The synthesis of creatine from glycine has been studied, and the techniques are ready for preparation of creatine labeled with N^{15} in the glycine moiety. This product can then be fed to the dog, thus labeling body creatine to make possible study of its metabolic course.

Significance to NIAMD research: Since creatine phosphate occupies a place of some importance in intracellular energy interchanges of the mammalian body, and since several hormones (e. g., androgens, estrogens, adrenocortical steroids with their pituitary colleagues) are known to affect

creatine-creatinine economy, further knowledge of factors and mechanisms of the systems involved will contribute basically to our understanding of metabolic disorders.

Proposed course of project: Creatine labeled with N^{15} in the glycine moiety will be synthesized and fed, in order to label body creatine before spinal cord transection. Determination of the N^{15} content of urinary creatine and creatinine in such animals should result in direct knowledge of the source of the creatinuria seen after cord transection. Abundance of N^{15} in muscle creatine and creatinine will also be determined.

With experience thus gained, similar studies can be carried out for transection of the midbrain.

It is also anticipated that later studies of the modification of creatine metabolism by hormone administration or withdrawal will be of value.

Budget Data
and
Honors, Awards, and Publications Sheet

10. NIAMD-23
SERIAL NUMBER

12.
BUDGET ACTIVITY: Research

13.
IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 OR 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Georgetown University
Walter Reed

14. No parallel research.

16.
LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

The Influence of the Central Nervous System on Metabolism and Endocrine Activity as Based on Transection of the Brain Stem in Dogs. Evelyn Anderson, Kathryn Knowlton, William T. Spence S. M. McCann, Gert L. Laqueur, David McK. Rioch, and Webb Haymaker Acta Neurovegetativa XII, pp. 53-94, 1955.

17. No honors and awards.

Analysis of Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases — INSTITUTE
2. Biochemistry and Nutrition — LABORATORY OR BRANCH
3. Fractionation and Isolation — SECTION
4. _____ — LOCATION
5. NIAMD-24 — SERIAL NO.
6. Studies on folic acid — PROJECT TITLE
7. M. Silverman and J. C. Keresztesy — PRINCIPAL INVESTIGATOR(S)
8. S. Futterman and R. C. Gardiner — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION:

Studies on folic acid:

(a) Naturally-occurring forms of,
(b) Enzymatic degradation of citrovorum factor and folic acid,
(c) The urinary compounds associated with folic acid metabolism in the rat.

Objectives: Folic acid, a vitamin of the B complex, has been shown to be needed by most animal species. Its importance and role in the enzyme systems which are related to growth, such as purine synthesis, have become better recognized in the past year. By identifying the natural-occurring forms of folic acid, it will be possible to obtain new anti-folic compounds, other than those used at present to control abnormal growth, specifically leukemia. Similarly, by identifying the products associated with folic acid metabolism in vivo and in vitro, a better understanding of some of the fundamental processes of growth will result.

Methods employed:

(a) The microbiologically-inactive form of folic acid in liver, hereafter referred to as prefolic acid, can be converted to the active citrovorum factor form by incubation with whole liver or an enzyme preparation obtained from liver. The problem consists of several interrelated parts which include purification of the enzyme which converts prefolic to citrovorum factor by usual protein fractionation methods and development of a fractionation process for the isolation of prefolic acid suitable for large-scale operation.

(b) Fractionation of liver extract for the purification of the enzyme which destroys citrovorum factor in the presence of l-glutamic and is being carried out using protein fractionation methods.

(c) The isolation of the substance believed to be related to isoglutamine in the urine of rats on a folic acid deficient diet is continuing, using solvent and chromatogram techniques.

(d) Customary enzymatic procedures are being employed to establish the necessary conditions under which soluble liver extracts degrade folic acid.

Major findings: Protein fractions have been prepared from horse liver which are active in converting prefolic acid to citrovorum factor. Thus far, partial purification of pork liver has produced enzyme preparations with low blank values which make the assay procedure much more sensitive. With such enzyme preparations requirements for histidine were demonstrated. The former requirement was specific and the latter non-specific.

A very critical method for the extraction of prefolic acid from horse liver in excellent yield was developed and applied to large-scale operation. Further purification of this crude extract was accomplished by adsorption and elution from charcoal and fractional solvent precipitations.

It has been shown that, in addition to the enzyme system present in soluble liver extracts, manganese, adenosinetriphosphate, citrate, and diphosphopyridine nucleotide are required for the conversion of folic acid to p-aminobenzoic acid and a free pteridine.

(In collaboration with F C. Ebaugh, Jr.) A metabolite of folic acid, which supports the growth of Leuconostoc Citrovorum has been obtained from human urine in a form which is from 60 to 80% pure. The product is a reduced and formylated derivative of folic acid whose properties (microbiological, spectrophotometric and stability) are identical with those of anhydro-citrovorum factor, a 5-10 bridge compound.

NIAMD-24
SERIAL NO.

Significance to Arthritis and Metabolic Research: The isolation and identification of the various metabolites of folic acid will result in a better understanding of biochemistry of such specific diseases as some of the anemias and leukemias. The mechanisms by which folic acid derivatives influence the formation of fundamental components of all living cells, the purines, are being widely studied. This project is directed at discovering how the various forms of folic acid exist in the body and how they are changed from inactive to active forms.

Proposed course of project: The following are the goals for the coming year:

(1) Completion of the purification of the reduced and formylated derivative of folic acid which occurs in human urine.
(2) Purification of the DPN dependent enzyme system which inactivates folic acid and identification of the pteridine end-product. Extension of these studies to formylated and reduced derivatives of folic acid.
(3) Continuation of attempts to isolate isoglutamine-like substance in urine from folic acid deficient rats.
(4) Purification of the enzyme which converts prefolic acid to citrovorum factor.
(5) Isolation of prefolic acid.

Analysis of Program Activities

Budget Data
and
Honors, Awards, and Publications Sheet

10. NIAMD-24
SERIAL NO.

12. -
BUDGET ACTIVITY: Research

13. No cooperating units.

14. No parallel research in PHS.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

On the occurrence of N^{10} formyltetrahydrofolic acid by enzymatic formylation of tetrahydrofolic acid and on the mechanism of this reaction. Biochim. Biophys. Acta 17, 589-591 (1955). G. R. Greenberg, L. Jaenicke and M. Silverman.

17. No honors and awards.

Analysis of Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY OR BRANCH

3. Fractionation and Isolation
SECTION

4. _____
LOCATION

5. NIAMD-25
SERIAL NO.

6. Large-scale processing of biological material
PROJECT TITLE

7. J. C. Keresztesy
PRINCIPAL INVESTIGATOR

8. _____
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION: Large-scale processing of biological material to extract and purify substances of biological importance.

Objectives: Many problems of importance in the biochemistry of disease require the isolation and identification of substances which are present in only trace amounts in the particular biological source. It becomes necessary, in order to obtain sufficient quantities of the desired compound for study, to process large amounts of biological source materials, such as liver, brains, body excretory products, plant materials, etc.

Methods employed: The laboratory is equipped with large-scale apparatus, such as stills, filters, reaction and extraction kettles, etc. In most isolation problems the original small-scale process has to be modified and developed, so that it can be carried out efficiently on the larger scale. This adaptation or process development is an important function of the laboratory. The degree of participation of the laboratory varies according to the needs of the specific problems.

Major findings: Among the more important operations have been the following: 108 kilos yeastamine processed for Factor 3 concentrate (Dr. K. Schwarz); 437 kilos rhododendron leaves, 502 kilos of various plant products and 179 kilos of veal brains for Natural Products Lab. (N.H.I); 98 kilos horse liver for prefolic acid, and 50 kilos human fecal matter for steroids (Dr. Heftmann).

Significance to Arthritis and Metabolic Research: Ordinary laboratories lack facilities to effectively carry on studies which require the extraction and processing of large quantities of biological materials. However, with the equipment and trained personnel of the large-scale laboratory, such investigations as the isolation of Factor 3, can be undertaken and successfully completed.

Proposed course of project: It now appears that the facilities for the safe handling of volatile and toxic solvents will be completed during the coming year. Many projects requiring this type of laboratory which have been delayed will be carried out.

Budget Data
and
Honors, Awards, and Publications Sheet

10. NIAMD-25
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 OR 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Laboratory of Chemistry of Natural Products NHI ___.

14. No parallel research in PHS.

16. No publications.

17. No honors or awards.

Analysis of Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases — INSTITUTE
2. Biochemistry and Nutrition — LABORATORY OR BRANCH
3. Fractionation and Isolation — SECTION
4. _____ — LOCATION
5. NIAMD-26 — SERIAL NO.
6. Microbiological assays for vitamins and amino acids — PROJECT TITLE
7. M. Silverman — PRINCIPAL INVESTIGATOR
8. H. Bakerman, M. Romine — OTHER INVESTIGATORS
9. Objective: The determination of the concentration of various vitamins and amino acids in a variety of materials, such as extracts and concentrates, foodstuffs, body tissues and excretory products, can be carried out by the use of microbiological assays. Under this project samples submitted by investigators on nutritional and allied projects are analyzed for their content of the specific vitamin or amino acid under study.

Methods employed: The use of microbiological assays for the determination of folic acid continues to be of major importance in the studies on folic acid. For example, with suitable test conditions one can distinguish between folic acid and its reduced derivatives. Further, the concentrations of 5- and 10-formyl derivatives of tetrahydrofolic acid in milli-microgram amounts can be determined in the presence of each other.

Significance to Arthritis and Metabolic Research: These microbiological assay procedures are a very important tool for the measurement of the concentrations of various cell constituents, i. e., amino acids and vitamins.

The following collaborative studies have been pursued in the past year:

(1) With Dr. J. M. Hundley
The influence of dietary rice on the concentration of arginine, lysine, and threonine in plasma and urine of humans.

(2) With Dr. A. E. Shaefer (ICNND)
The influence of high concentrations of sea salt (Far East diets) on the stability of riboflavin and pantothenic acid during cooking.

(3) With Dr. O. Mickelsen
The destructive effect of exposure to ethylene oxide on niacin, riboflavin, folic acid, pantothenic acid and biotin.

(4) With Dr. J. Field
The excretion of riboflavin and pantothenic acid by diabetic humans; the influence of insulin on these excretion patterns.

(5) With Dr. F. Ebaugh Jr.
The excretion of "labile" citrovorum factor in human urine under normal and pathological conditions.

Proposed course of project: With the extension of laboratory findings to clinical investigations, there will be an increasing use of these assays from clinical materials.

Budget Data
and
Honors, Awards, and Publications Sheet

10. NIAMD-26
SERIAL NO.

12.
BUDGET ACTIVITY: Research

13. No cooperating units.

14. No parallel research in PHS

16. No publications.

17. No honors and awards.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY OR BRANCH

3. Enzymes & Cell.Biochem.
SECTION

4.
LOCATION (IF OTHER THAN BETHESDA)

5. NIAMD-27
SERIAL NO.

6. Studies on the structure, biosynthesis and intermediary metabolism of nucleic acids and nucleotides.
PROJECT TITLE

7. Dr. Leon A. Heppel
PRINCIPAL INVESTIGATOR(S)

8. Russell Hilmoe
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objective of this project is to discover pathways for the biosynthesis and breakdown of nucleic acids and smaller polynucleotides.

Methods Employed: With enzymes purified from suitable sources various biosynthetic and degradative reactions are carried out. The products are studied by means of paper chromatography, paper electrophoresis, chemical analysis, ion-exchange column chromatography and isotope tracer methods.

Major Findings: The first successful enzymatic synthesis of dinucleotides and larger polynucleotides from small molecules such as cyclic mononucleotides was previously demonstrated by us, using pancreatic ribonuclease. The synthesis involved an exchange or transfer reaction. If this mechanism is of wide significance in nucleic acid metabolism, then enzymes like pancreatic ribonuclease must occur in other tissues. With Dr. Kaplan, we have now discovered enzymes in calf liver and spleen with exactly the same specificity and heat stability as the pancreatic enzyme, and extensive purification has been carried out.

In June, 1955, an investigation was begun in collaboration with Dr. S. Ochoa on the structure of certain polymers derived from nucleoside 5'-diphosphates by the action of an enzyme purified from

9. PROJECT DESCRIPTION - Continued

the bacterium, Azotobacter Vinelandii. This investigation has established that the polymers are large molecules which behave chemically and enzymatically exactly like ribonucleic acid, except for the distribution of the nitrogenous bases. With these synthetic polynucleotides we have been able to show for the first time that pancreatic ribonuclease cleaves a pyrimidine polynucleotide chain in random fashion to form a series of small polynucleotides 2-5 units in length with cyclic end groups. We also demonstrated the transfer or exchange mechanism with synthetic polymers. Furthermore we have made observations on the mechanism of polymer synthesis.

Perhaps the most significant recent discovery was the finding of a new class of small ribopolynucleotides. They were formed by an enzyme which we purified from liver nuclei acting on the adenylic acid synthetic polymer. These new small polynucleotides contain 5'-phosphomonoester endgroups in contrast to all previously obtained small polynucleotides which have phosphomonoester groups at C 3'. The importance of these new compounds lies in the fact that they appear to be active intermediates in polynucleotide biosynthesis.

Studies on the mechanism of nucleases using O^{18} are being continued.

Significance to NIAMD Research: It is expected that these studies will provide information on the mechanisms of nucleotide and nucleic acid synthesis and breakdown. The importance of these substances in cell economy is emphasized by the great attention now being paid to them by investigators in all fields of biology and medicine.

Proposed Course of Project: Further studies on the mechanism of synthesis of the Azotobacter polymers is contemplated. We also plan to study nucleic acid synthesis in animal tissues. Present evidence indicates that the liver nuclei fractions are carrying out a phosphorolysis of polynucleotide, similar to the synthetic reactions of the Azotobacter system. The mechanism of action of nucleases and diesterases will be further studied.

1955

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-27
SERIAL NUMBER

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 OR 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10).

Dr. D. E. Koshland Jr., Brookhaven National Laboratory
Dr. S. Ochoa, Department of Biochemistry, New York University
Dr. Henry S. Kaplan, Commonwealth Fellow, on Sabbatical leave from Stanford University, until July 1955.

14.

No parallel research in the P. H. S.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-27
SERIAL NUMBER

16. LIST OF PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Nucleotide Exchange Reactions Catalysed by Ribonuclease and Spleen Phosphodiesterase. 1. Synthesis and interconversion of simple esters of ribomononucleotides, by L. A. Heppel and P. R. Whitfeld, The Biochemical Journal, 60, 1 (1955).

Nucleotide Exchange Reactions Catalysed by Ribonuclease and Spleen Phosphodiesterase. 2. Synthesis of polynucleotides, by L. A. Heppel, P. R. Whitfeld and R. Markham, The Biochemical Journal, 60, 8 (1955).

The Enzymic Hydrolysis of Ribonucleoside-2':3' Phosphates, by P. R. Whitfeld, L. A. Heppel and R. Markham, The Biochemical Journal, 60, 15 (1955).

A Note on the Structure of Triphosphopyridine Nucleotide, by L. A. Heppel, P. R. Whitfeld and R. Markham, The Biochemical Journal, 60, 19 (1955).

Contributions to "METHODS IN ENZYMOLOGY" edited by Colowick and Kaplan:

Separation of Proteins from Nucleic Acids, by Leon Heppel, Vol. 1, 137 (1955).

Xanthine Oxidase from Milk, by B. L. Horecker and L. A. Heppel, Vol. II, 482 (1955).

Intestinal Phosphomonoesterase, by Leon A. Heppel, Vol. II, 530 (1955).

"5" Nucleotidases, by Leon A. Heppel and R. J. Hilmoe, Vol. II, 546 (1955).

Inorganic Pyrophosphatase from Yeast, by Leon A. Heppel, Vol. II, 570 (1955).

Small Polyribonucleotides with 5'-phosphomonoester End-groups, by Leon A. Heppel, Priscilla J. Ortiz and Severo Ochoa, Science, in press.

17. No honors or awards.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD — INSTITUTE
2. Biochemistry and Nutrition — LABORATORY OR BRANCH
3. Enzymes & Cell.Biochem. — SECTION
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NIAMD-28 — SERIAL NO.

6. Carbohydrate metabolism in the mammalian spleen
PROJECT TITLE

7. Dr. Gilbert Ashwell
PRINCIPAL INVESTIGATOR(S)

8. Jean Hickman
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Earlier work in this laboratory on the metabolism of ribose-5-phosphate in mouse spleen extracts had established the presence of two new sugar phosphates (D-xylulose phosphate and 3-ketopentose phosphate). A study of the enzyme systems involved was undertaken in the hope of elucidating the role these compounds might play in mammalian carbohydrate metabolism.

Methods employed: The enzyme purification proceedures involve the use of heat, pH adjustment, gel adsorption and acetone fractionation. Assay methods include colorometric and spectrophotometric sugar determinations. Isolation of intermediates is accomplished by the use of column and paper chromatography.

Major Findings: Extensive effort has been devoted to the isolation of an enzymatic system responsible for the formation and utilization of 3-ketopentose phosphate. However, due to the lability and the extremely small yield of this compound, progress has been slow and this phase of the problem has been temporarily shelved.

Purification of the enzyme system responsible for the formation of xylulose phosphate from ribulose phosphate has resulted in an enzyme preparation approximately 200-fold purified over the original spleen homogenate. This preparation, which is free from isomerase and transketolase, appears to have significantly different properties from the more highly purified enzyme (phosphoketopentose epimerase) isolated from bacterial sources (Stumpf, Hurwitz, Horecker).

9. PROJECT DESCRIPTION - Continued

Significance to NIAMD Research: It is expected that study of the function of these compounds and of the pathways by which they are utilized will contribute to our knowledge of the overall metabolic process and thus lead to a more rational approach to the understanding and treatment of the pathological alterations in metabolism.

Proposed Course of Project: The very recent discovery of the key role of xylulose phosphate in pentose metabolism by E. Racker (New York) and J. Hurwitz and B. L. Horecker (NIH) has resolved one important part of the problem outlined above. Further purification and study of the properties of the epimerase isolated from mammalian spleen will permit a determination of the mechanism of the reaction and the isolation of possible intermediates. Further information on the possible role of 3-ketopentose phosphate in pentose metabolism may also result.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-28
SERIAL NUMBER

12. BUDGET ACTIVITY: Research

13.

No cooperating units.

14.

No parallel research in the P.H.S.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-28
SERIAL NUMBER

16. LIST OF PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Identification of 3-ketopentose arising from Ribose Phosphate, by Gilbert Ashwell and Jean Hickman, J. Am. Chem. Soc., 77, 1062 (1955).

Colorometric Determination of Sugars, by Gilbert Ashwell, in "METHODS IN ENZYMOLOGY", edited by Colowick and Kaplan, Vol. III, In press.

Inhibition of the Anaerobic Glycolysis in Pigeon Hemolysates by Multivalent Anions, by Z. Dische and G. Ashwell, Biochim. et Biophys. Acta 17, 56 (1955).

17. No honors and awards.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY OR BRANCH

3. Enzymes & Cell. Biochem.
SECTION

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5 NIAMD-29
SERIAL NO.

6. Metabolism and Biological Function of Uridine Nucleotides in Cells of Normal and Pathological Organisms.
PROJECT TITLE

7. Dr. Herman M. Kalckar
PRINCIPAL INVESTIGATOR

8. Dr. Elizabeth Maxwell
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: To pursue the studies of last year on the biological function of complex glucose, galactose and glucuronide compounds in the living cell. The main emphasis has been placed on the biosynthesis and metabolism of uridine diphosphogalactose ('active' galactose).

One of the objectives was to use our knowledge gathered from the last year's studies and our recently designed micromethod of high specificity for galactose compounds for a study on a familial disease in man (especially infants), called galactosemia or galactose diabetes. Our objective was to try to decide whether this disease is due to minor changes in the rates of several reactions of the general carbohydrate metabolism or largely to a more direct genetic block of one of the enzymes which catalyzes the metabolism of galactose-1-phosphate.

Methods Employed: Extensive use was made of paper chromatography and enzymatic spectrophotometry. New methods were developed for enzymatic synthesis and analysis of compounds like radioactive uridine diphospho glucose (* C^{14} in glucose part) or uridine diphospho galactose. These methods were put to use in analysis of blood specimen from children.

9. PROJECT DESCRIPTION - Continued

Major Findings: The author, in collaboration with Drs. E. Anderson and Dr. Kurt Isselbacher, found that red blood cells from galactosemic children are completely devoid of the enzyme which incorporates galactose-1-phosphate into uridine nucleotides. The enzyme is always found in normal children or in adults with diseases other than galactosemia, such as milk allergy. A number of other enzymes such as the galactose phosphorylating enzyme (galactokinase) or the glucose-1-phosphate incorporating enzyme, forming uridine diphospho glucose, are in the blood cells of galactosemic children. The enzyme defect seems therefore to be highly specific.

Dr. E. Maxwell has been purifying galacto-waldenase, the enzyme responsible for the conversion of galactose to glucose (as the nucleotides). This purification has not only enabledus to study galactose metabolism by superior methods than hitherto used, but the study of the enzyme itself is of great interest. The cofactor requirement is under study.

Drs. Anderson and K. Kurahashi have purified the enzyme which catalyzes the incorporation of galactose-1-phosphate into uridine nucleotides. (This is the enzyme which is defective in galactosemic subjects). Dr. Kurahashi has developed an enzymatic spectrophotometric method for galacto kinase in blood.

In joint work with Dr. R. Burton (NINDB) the metabolism of galactose phosphate in brain and mammary gland was investigated. This study revealed that, unlike liver, neither growing brain tissue (from newborn rat) nor the lactating mammary gland seem to be able to utilize administered galactose for the biosynthesis of brain galacto lipids and for lactose, respectively.

Dr. Robert Wheat is studying the metabolism of uridylate bound N-acetyl glucosamine in an E. coli strain which generates an interesting antigen (v.i. antigen). The V. i. antigen is composed of N-acetyl 2 amino glucuronic acid. It is of great interest that Dr. Wheat has found a specific transfer and presumably oxidation of nucleotide bound N-acetyl glucosamine; a strongly fluorescent compound is formed which may be a precursor of the antigen.

Significance to NIAMD Research: The interest in the observations of the specific enzyme defect in galactosemic children may be many-fold. A better understanding of the nature of heritable metabolic diseases in man might well be developed by a continued pursuit of the above mentioned galactosemic project. The block also seems to indicate which pathway is the main course of intermediary galactose metabolism in man. The methods developed might be used in family studies to discover traits as well as an added tool in the diagnosis of a disease in which early diagnosis is of such critical importance. Since the galactose

9. PROJECT DESCRIPTION - Continued

tolerance test is not without hazards in galactosemic children, our methods have several advantages. It is necessary, however, to ascertain on a larger group of subjects than the 8 cases which we have reported that the new method applies to all cases of galactosemia as disgnosed by the old method. The pathogenesis of tissue damage including cataract might be directly related to the alteration in the metabolism of galactose.

The study of the galacto-waldenase might be of particular interest for a study of the synthesis of brain constituent and brain function as well as for an understanding of the physiology of lactation.

The study on the specific metabolism of uridylic bound N-acetyl glucosamine in a microorganism which produces V.i. antigon which is such a common cell wall constituent in the highly virulent microorganism of the typhoid group could contribute to our knowledge about the factors involved in virulence of microorganism. The transfer reactions here involved may also tell us something about the nature of cell wall synthesis in general and hence about the nature of action of our most important antibiotics.

Proposed Course of Project: We are planning a further study of the disease galactosemia, especially in order to see whether the missing transfer enzyme is replaced by an abnormal enzyme.

A study on purified galacto-waldenase with special reference to cofactors and the nature of the inversion. The latter should also be approached by means of isotopes, especially tritium.

A study of the biosynthesis of complex galactosides, aminosugars and other constituents of the cell wall.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-29
SERIAL NUMBER

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 OR 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10).

Dr. Elizabeth Anderson, Postdoctoral Fellow of the American Cancer Society
Dr. Robert W. Wheat, Postdoctoral Fellow, USPHS
Dr. Kiyoshi Kurahashi, Postdoctoral Fellow, Jane Coffin Childs Memorial Fund, since November 1, 1955.
Miss Bodil Waage-Jensen, Laboratory Assistant, Eli Lilly Grant, Administered by American-Scandinvian Foundation, since March 1,1955.
Dr. Kurt J. Isselbacher, NIAMD Arthritis and Rheumatism Branch
Dr. Robert Main Burton, National Institute of Neurological Diseases and Blindness, Research Branch.

14.

No parallel research in the P.H.S.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-29
SERIAL NUMBER

16. LIST OF PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Uridyl Transferases, their occurrence and physiological role, by Agnete Munch-Petersen, H. M. Kalckar and Evelyn E. B. Smith, Kgl. D. Vidansk. S.; Dan. Biol. Medd. 22, No. 7 (1955).

Galacto-waldenase and the enzymic incorporation of Galactose-1-Phosphate in Mammalian Tissues, by Elizabeth Maxwell, H. M. Kalckar and Robert Main Burton, Biochimica et Biophysica Acta, 18, 389 (1955).

Enzymatic Micro Determinations of Uric Acid, Hypoxanthine, Xanthine, Adenine and Xanthopterine by Ultraviolet Spectrophotometry, by Paul Plesner and Herman M. Kalckar. Methods of Biochemical Analysis, in press.

The Hydrolysis of Purine and Pyrimidine Nucleoside Triphosphates by Myosin, by W. Wayne Kielley, H. M. Kalckar and L. B. Bradley, J. Biol. Chem., in press.

Enzymatic Synthesis and Metabolism of Uridinediphospho Glycosyl Compounds, by H. M. Kalckar, Elizabeth Anderson and A. Munch-Petersen, Pubblicazioni della Stazione Zoologica di Napoli (Jubilee Volume honoring Professor Dohrn), in press.

Enzymatic Determination of UTP, by Herman M. Kalckar and Elizabeth Anderson. Methods in Enzymology, Vol. III, in press.

Determination of UDPG by means of UDPG Dehydrogenase, by Elizabeth S. Maxwell, Herman M. Kalckar and J. L. Strominger. Methods in Enzymology, Vol. III, in press.

Galactosemia, A Congenital Defect in a Nucleotide Transferase, A Preliminary Report, by Herman M. Kalckar, Elizabeth P. Anderson and Kurt J. Isselbacher. Proceedings National Academy of Sciences, in press.

Galactosemia, A Congenital Defect in a Nucleotide Transferase, by Herman M. Kalckar, Elizabeth P. Anderson and Kurt J. Isselbacher, Biochim. et Biophys. Acta, in press.

17. No honors or awards.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY OR BRANCH

3. Enzymes and Cellular Biochemistry
SECTION

4.
LOCATION (IF OTHER THAN BETHESDA)

5. NIAMD-30
SERIAL NUMBER

6. Studies on the Biosynthesis and Degradation of Purines
PROJECT TITLE

7. Jesse C. Rabinowitz
PRINCIPAL INVESTIGATOR

8. W. E. Pricer, Jr.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: The objective of this project is to determine biological mechanisms involved in the metabolism of purines.

Methods Employed: Compounds involved in purine metabolism have been synthesized containing radioactive carbon. With whole cells and enzyme preparations of microbial origin, the products of purine metabolism have been identified using isotopic tracer methods, paper chromatography, and specific chemical and enzymatic assays.

Major Findings: The major findings of this project during the past year have been concerned with the metabolism of formiminoglycine. The products formed from formiminoglycine in fermentations by whole cells are acetic acid and carbon dioxide. The specific carbon atoms of formiminoglycine which act as precursors of these products have been determined using radioactive tracers, and the results indicate that one-carbon intermediates are involved. Glycine is not metabolized by the cells unless catalytic amounts of formiminoglycine are added. Cell extracts have been obtained which convert formiminoglycine to glycine and formic acid. After treating the extract with an anion exchange resin, this process becomes dependent on the addition of some factor present in a boiled extract of the organism. The factor can be replaced by folic acid or various related pteridine derivatives.

9. PROJECT DESCRIPTION - continued

Significance to NIAMD Research: Purines are synthesized and are degraded to uric acid and other products by living organisms; they are involved in energy transfer reactions in the form of nucleotides; they are components of nucleic acids and are found in all living cells. The metabolism of purines is therefore of fundamental importance in all living organisms. Knowledge of the enzymatic reactions involved in their biosynthesis, however, is fragmentary. The metabolism of purines in microbial systems and the identification of the compounds involved has been a fruitful means of disclosing metabolic pathways and organic compounds previously unknown.

The metabolism of one-carbon compounds (formate, formaldehyde, etc.) remains poorly defined at the present time and many laboratories are interested in this problem. The present system offers a new approach to the subject which may provide useful and badly needed information.

Proposed Course of Project: Problems which shall be investigated during the next calendar year include
1) the identification of the compounds and enzymatic reactions involved in the conversion of formiminoglycine to acetic acid and carbon dioxide by way of glycine;
2) the utilization of the microbial enzyme systems for the complete degradation and isolation of each carbon and nitrogen atom of the purine molecule; and
3) an evaluation of the general occurrence and significance in other biological systems of the compounds which have been encountered in these studies.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-30
SERIAL NUMBER

12. BUDGET ACTIVITY:
Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S)

Dr. N. P. Salzman, Laboratory of Infectious Diseases,
National Microbiological Institute

14.

No parallel research in the P.H.S.

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-30
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Purine Fermentation by Clostridium cylindrosporum. III. 4-Amino-5-Imidazolecarboxylic Acid and 4-Aminoimidazole. By Jesse C. Rabinowitz, J. Biol. Chem., in press.

Purine Fermentation by Clostridium cylindrosporum. IV. 4-Ureido-5-Imidazolecarboxylic Acid. By Jesse C. Rabinowitz and W. E. Pricer, Jr., J. Biol. Chem., in press.

17.

No honors or awards.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY OR BRANCH

3. Enzymes & Cell.Biochem.
SECTION

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. NIAMD-31
SERIAL NO.

6. The enzymatic transformation of carbohydrates and their intermediary metabolism.
PROJECT TITLE

7. Dr. B. L. Horecker
PRINCIPAL INVESTIGATOR(S)

8. Miss P. Z. Smyrniotis
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To provide further information on the new pathway of carbohydrate metabolism in order to define the role of these reactions and intermediates in the chemistry of the cell.

Methods employed: With enzymes purified from suitable sources the individual reactions are studied in detail and reaction products isolated and identified. These products are then employed as substrates for succeeding steps. Where possible, these compounds are synthesized with C^{14} or P^{32} and the reactions studied in intact cells or unfractionated tissue preparations.

Major Findings: Previous work by Calvin at the University of California and from this laboratory suggested that pentose phosphate played an important role in photosynthesis. This has now been confirmed and the pathway of carbohydrate synthesis from CO_2 completely clarified. The following sequence of reactions has been shown to occur:

9. PROJECT DESCRIPTION - Continued

1) 6 RuDP + 6 CO_2 $\longrightarrow$ 12 PGA
2) 12 PGA + 12 ATP + 12 DPNH $\rightleftharpoons$ 12 G-3-P + 12 P + 12 DPN + 12 ADP
3) 10 G-3-P $\rightleftharpoons$ 5 FDP
4) 5 FDP $\longrightarrow$ 5 F-6-P + 5 P
5) 2 F-6-P + 2 G-3-P $\rightleftharpoons$ 2 E-4-P + 2 Xu-5-P
6) 2 F-6-P + 2 E-4-P $\rightleftharpoons$ 2 S-7-P + 2 G-3-P
7) 2 S-7-P + 2 G-3-P $\rightleftharpoons$ 2 Xu-5-P + 2 R-5-P
8) 2 R-5-P $\rightleftharpoons$ 2 Ru-5-P
9) 4 Xu-5-P $\rightleftharpoons$ 4 Ru-5-P
10) 6 Ru-5-P + 6 ATP $\longrightarrow$ 6 RuDP + 6 ADP

Sum: 6 CO_2 + 18 ATP + 12 DPNH $\longrightarrow$ F-6-P + 17 P + 18 ADP + 12 DPN

RuDP = ribulose diphosphate
PGA = phosphoglyceric acid
G-3-P = phosphoglyceraldehyde
FDP = fructose diphosphate
F-6-P = fructose 6-phosphate
E-4-P = erythrose 4-phosphate
Xu-5-P = xylulose 5-phosphate
Ru-5-P = ribulose 5-phosphate
R-5-P = ribose 5-phosphate
S-7-P = sedoheptulose 7-phosphate

On the basis of these reactions and from the ATP and DPNH requirements, it can be predicted that the maximum efficiency of photosynthesis is 6 quanta per mole of CO_2. This is considerably less than proposed by Burk and Warburg, but in agreement with the data of most workers in this field.

In connection with this work several enzymes, including phosphoribulokinase (step 10), phosphoriboisomerase (step 8), and epimerase (step 9), were purified and the carboxylation enzyme (step 1) was obtained in essentially pure form. A new method has been developed for the preparation of ribulose diphosphate and xylulose 5-phosphate. Both of these intermediates have now been obtained free of other esters.

It has now been determined that the pentose involved in the transketolase reaction is D-xylulose 5-phosphate, rather than D-ribulose 5-phosphate. These results, obtained independently by Racker and his associates at the Public Health Institute of New York, indicate that the xylulose ester, discovered earlier by Ashwell, is important in nucleic acid formation and carbon dioxide synthesis.

In $C^{14}O_2$ incorporation into glycogen in liver slices, little $C^{14}O_2$ appears in position 2. This suggests that under these experimental conditions there is not an extensive oxidation of carbohydrate by the phosphogluconate pathway. In slices from fed animals or when glycerol is added to the medium, there

9. PROJECT DESCRIPTION - Continued

is a dilution of isotope in carbon atom 4. This is consistent with previous suggestions from other laboratories that a complete equilibration of dihydroxyacetone phosphate and glyceraldehyde phosphate does not occur under these conditions.

The metabolism of erythrose was studied with a strain of Alciligenes faecalis isolated from soil. This organism can be adapted to the tetrose as the sole carbon source. Evidence was obtained for erythrose 4-phosphate as the first step in the process.

Preliminary work has been done on a method for the isolation of desoxyribose from tissue DNA. This method will be applied to a study of the biological origin of desoxypentose.

Significance to NIAMD Research: The importance of the pentose phosphate pathway of carbohydrate metabolism to the basic economy of the cell has been confirmed and extended. It is hoped ultimately to develop a complete understanding of the role of these reactions in cell growth and the synthesis of important constituents such as amino acids, DNA and RNA. This knowledge would provide a basis for the comparison of normal and diseased tissue.

Proposed Course of Project: The detailed mechanism of many of these reactions remains to be elucidated. Further efforts to isolate erythrose 4-phosphate will be made, with particular emphasis on the bacterial system. Studies of the origin of desoxyribose will be made in order to provide a basis for the isotope work in progress. In collaboration with Dr. Eagle of the Microbiological Institute, studies will be carried out on the metabolism of glucose in cells in tissue culture.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-31
SERIAL NUMBER

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

A. Weissbach, - Postdoctoral Fellow, National Foundation for Infantile Paralysis - until August 1955

J. Hurwitz, - Postdoctoral Fellow, American Cancer Society

P. Stumpf, - Postdoctoral Fellow, USPHS, until June 1955

H. Hiatt, - NIAMD, Clinical Investigations, until June 1955

Y. Takagi, - Postdoctoral Fellow, Jane Coffin Childs Memorial Fund, since Sept. 1, 1955

E. Heath, - Postdoctoral Fellow, American Heart Association, since Sept. 1, 1955

H. Eagle, - NMI, LID, Section on Experimental Therapeutics

14.

No parallel research in the PHS

Analysis of NIH Program Activities

Honors, Awards, and Publication Sheet

15. NIAMD-31
SERIAL NUMBER

16. LIST OF PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Tetrose Phosphate and Sedoheptulose Diphosphate Formation, by B. L. Horecker, P. Z. Smyrniotis, H. Hiatt and P. Marks. J. Biol. Chem., 212, 827 (1955).

Purification and Properties of Yeast Transaldolase, by B. L. Horecker and P. Z. Smyrniotis. J. Biol. Chem., 212, 811 (1955).

Carbohydrate Metabolism, by B. L. Horecker and Alan H. Mehler. Book: Annual Review of Biochemistry, Vol. 24, 207 (1955).

The Formation of Glycine from Ribose-5-phosphate, by A. Weissbach and B. L. Horecker. Amino Acid Metabolism, The Johns Hopkins Press, Baltimore, (1955).

Pathways of Carbohydrate Metabolism in Microorganisms, by I. C. Gunsalus, B. L. Horecker and W. A. Wood. Bact. Review, 19, 79 (1955).

Contributions to "METHODS IN ENZYMOLOGY" edited by Colowick and Kaplan:

Glucose-6-phosphate Dehydrogenase and 6-Phosphogluconic Dehydrogenase, by A. Kornberg, B. L. Horecker and P. Z. Smyrniotis, Vol. 1, 323 (1955).

Transaldolase, by B. L. Horecker and P. Z. Smyrniotis, Vol. 1, 381 (1955).

Transketolase, by B. L. Horecker and P. Z. Smyrniotis, Vol. 1, 371 (1955).

TPNH Cytochrome c Reductase (Liver), by B. L. Horecker, Vol. II, 704 (1955).

Xanthine Oxidase from Milk, by B. L. Horecker and L. A. Heppel, Vol. II, 482 (1955).

D-Glucose-6-phosphoric Acid, by B. L. Horecker, Vol. III, in press.

Preparation and Analysis of Ribose-5-phosphate, by B. L. Horecker, Vol. III, in press.

Preparation and Analysis of Heptulose Phosphates, by B. L. Horecker, Vol. III, in press.

The Orcinol Reaction for Mixtures of Pentose and Heptulose, by B. L. Horecker, Vol. III, in press.

16. LIST OF PUBLICATIONS - continued

Preparation and Analysis of Ribulose-5-phosphate, by B. L. Horecker, Vol. III, in press.

Preparation and Analysis of 6-Phosphogluconate, by B. L. Horecker Vol. III, in press.

Distribution of Radioactive Carbon Dioxide Incorporated into Rat Liver Glycogen, by P. A. Marks and B. L. Horecker, J. Biol. Chem., in press.

Erythrose Metabolism in a Strain of Alcaligenes faecalis, by Howard H. Hiatt and B. L. Horecker, J. Bact., in press.

The Preparation of Sedoheptulose Diphosphate, by P. Z. Smyrniotis and B. L. Horecker, J. Biol. Chem., in press.

Spinach Phosphoribulokinase, by B. L. Horecker and P. Z. Smyrniotis, J. Biol. Chem., in press.

The Enzymatic Synthesis and Properties of Ribulose-1,5-Diphosphate, by B. L. Horecker, J. Hurwitz and A. Weissbach, J. Biol. Chem., in press.

The Enzymatic Formation of Phosphoglyceric Acid from Ribulose Diphosphate and Carbon Dioxide, by A. Weissbach, B. L. Horecker and J. Hurwitz, J. Biol. Chem., in press.

The Role of Xylulose-5-phosphate in Xylose Metabolism of Lactobacillus pentosus, by P. K. Stumpf and B.L.Horecker, J. Biol. Chem., in press.

17.

No honors or awards.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY

3. Intermediary Metabolism
SECTION

4. _____

5. NIAMD-32
SERIAL NO.

6. Creatinuria in Thyrotoxicosis
PROJECT TITLE

7. Miss J. D. Benedict; Dr. Leonard Laster; Dr. D. Stetten, Jr.
PRINCIPAL INVESTIGATORS

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: The objective of this project is to study the mechanism by which thyrotoxicosis in the human produces creatinuria.

Methods Employed: N^{15}-labeled glycine is synthesized and administered orally to patients. The liver incorporates this substrate into creatine which is then converted to creatinine, to a greater or lesser extent, by muscle. Subsequently, these metabolic products together with hippuric acid are isolated from the urine, and the amounts of isotope they contain are determined and compared.

Patient Material:		No.	Average Stay in Days
Admissions:	Adult Males	2	21
	Adult Females	0	
	Children	0	
Outpatient:	No. of patients	0	
	No. of visits	0	

Major Findings: In both subjects initially far higher concentrations of isotope were found in creatine than in creatinine and the decline in isotope abundance was more rapid in creatine than in creatinine. It has been concluded that urinary creatine, in this disease, arises, not from muscle creatine but rather from freshly synthesized creatine formed chiefly in the liver.

Significance to NIAMD Research: Creatinuria is one of the principal biochemical manifestations of thyrotoxicosis. Therefore, studies of this defect may lead to a better understanding of the disease, and of the asthenia associated with it.

Proposed Course of Research: Further aspects of the problem of creatinuria in thyrotoxicosis will be explored.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-32
SERIAL NUMBER

12. Research
BUDGET ACTIVITY

13. NO COOPERATING UNITS

14. NO PARALLEL RESEARCH IN PHS

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-32
SERIAL NUMBER

16. PUBLICATIONS FROM THIS PROJECT:

The Origin of Creatine in Progressive Muscular Dystrophy, Jean D. Benedict, Helen J. Kalinsky, Louis A. Scarrone, Arthur R. Wertheim, and DeWitt Stetten, Jr., J. Clin. Invest., 34, 141 (1955).

17. NO HONORS OR AWARDS.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY

3. Intermediary Metabolism
SECTION

4. _____

5. NIAMD-33
SERIAL NO.

6. Studies on the Mechanism of Action of Transamidinase
PROJECT TITLE

7. Miss J. D. Benedict and Dr. D. Stetten, Jr.
PRINCIPAL INVESTIGATORS

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: To purify the enzyme transamidinase found in kidney tissue in order to study the mechanism of the reaction whereby arginine reacts with glycine to produce ornithine and glycocyamine.

Methods Employed: An assay system was developed which depends on the use of the Jaffe reaction for the determination of glycocyamine produced by the enzymatic reaction.

Major Findings: A preliminary attempt has been made to purify this enzyme using ammonium sulfate fractionation.

Significance to NIAMD: The synthesis of creatine is of interest because this compound is a precursor of phosphocreatine which is of importance in energy metabolism.

Proposed Course of Project: Further attempts will be made to purify this enzyme. The mechanism of the enzymatic reaction will then be studied using $C^{14}N^{15}$ labeled arginine.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-33
SERIAL NUMBER

12. Research
BUDGET ACTIVITY

13. NO COOPERATING UNITS

14. NO PARALLEL RESEARCH IN PHS

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

16. NO PUBLICATIONS

17. NO HONORS OR AWARDS

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY

3. Intermediary Metabolism
SECTION

4. _____

5. NIAMD-34
SERIAL NO.

6. Evaluation of Catabolic Pathways for Glucose in Intact Cells
PROJECT TITLE

7. Dr. Ben Bloom; Dr. Frank Eisenberg, Jr.
PRINCIPAL INVESTIGATORS

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objectives of this project are: (1) evaluation of the extent to which the Embden-Meyerhof pathway operates in the catabolism of glucose to CO_2 in various in vitro systems and in vivo; (2) a comparison of this quantity to that obtained in tissues from diabetic and fasted animals; (3) the further characterization of the non-Embden-Meyerhof pathways active in liver slices.

Methods Employed: The methods employed have involved determination of the radiochemical yield of carbon dioxide from various C^{14} labeled substrates in vitro and in vivo. A simplified technique for the analysis of C^{14} activities in carbons 1 and 6 of glucose has been used. Also, the isolation of fatty acids and assay for C^{14} by direct mounting has been a feature of this project.

Major Findings: With the aid of several newly developed equations it has been demonstrated that approximately 44% of glucose catabolism in liver slices derived from normal rats and rabbits proceeds via the glycolytic pathway, and that the remaining 56% of glucose catabolism is consistent with the requirements of the oxidative pathway. It has also been shown that diaphragm sections obtained from normal rats catabolize glucose exclusively through the glycolytic pathway, although this tissue is capable of oxidizing gluconic acid, a product of the oxidative pathway. Brain slices obtained from normal rabbits likewise oxidize glucose exclusively via the Embden-Meyerhof sequence. Furthermore, it has been observed that in liver slices derived from diabetic and fasted rats an increased fraction of glucose is glycolyzed as compared to that

catabolized non-glycolytically. No evidence to date has been obtained for the occurrence of any but the Embden-Meyerhof and phosphogluconate oxidation pathways in liver slices.

Significance to NIAMD Research: In order properly to evaluate the disturbances in glucose metabolism occurring in those pathological conditions characterized by abnormal carbohydrate metabolism, it is essential to understand the behavior of glucose in normal cells. The program outlined in this report should yield results which will facilitate such understanding.

Proposed Course of Project: Certain aspects of this problem will be pursued in vivo.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-34
SERIAL NUMBER

12. Research
BUDGET ACTIVITY

13. NO COOPERATING UNITS

14. NO PARALLEL RESEARCH IN PHS

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-34
SERIAL NUMBER

16. PUBLICATIONS FROM THIS PROJECT:

The Fraction of Glucose Catabolized via the Glycolytic Pathway, B. Bloom and D. Stetten, Jr., J. Biol. Chem., 212, 555 (1955).

Glucose Catabolism by Mammalian Tissues, B. Bloom, Proc. Soc. Exper. Biol. and Med., 88, 317 (1955).

The Hormones of the Islets of Langerhans, D. Stetten, Jr., and B. Bloom, The Hormones III, 175 (1955).

Glucose Catabolism in Liver Slices via the Phosphogluconate Oxidation Pathway, B. Bloom, F. Eisenberg, Jr., and D. Stetten, Jr., J. Biol. Chem., 215, 461 (1955).

Fraction of Glucose Catabolized via the Embden-Meyerhof Pathway: Alloxan Diabetic and Fasted Rats, B. Bloom, J. Biol. Chem., 215, 467 (1955)

Studies of the Metabolism of the Amidine Group of Arginine in the Intact Rat, D. Stetten, Jr., and B. Bloom, J. Biol. Chem., (In press).

17. None.
HONORS AND AWARDS

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY

3. Intermediary Metabolism
SECTION

4. _____

5. NIAMD-35
SERIAL NO.

6. Mechanism of Action of Enzymes
PROJECT TITLE

7. Dr. Yale J. Topper; Dr. Ben Bloom
PRINCIPAL INVESTIGATORS

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: The objective of this project is to study known enzymes with respect to the detailed chemical mechanism by which they effect transformations of their respective substrates.

Methods Employed: (1) Non-isotopic substrates are exposed to the biological catalysts in the presence of D_2O and the distribution of deuterium in the products is determined; (2) deuterated substrates are incubated with the enzymes and the redistribution of the isotope is then studied.

Major Findings: In a study of phosphoglucose isomerase derived from rabbit muscle it has been found that in the conversion of fructose-6-phosphate into glucose-6-phosphate, one atom of carbon-bound deuterium is incorporated into the product when the reaction is carried out in D_2O It was furthermore shown that the isotope resided on C-2 of the carbon skeleton. It appears on this basis, that the sequence of events involves an opening of the hemiacetal ring, followed by activation of the alpha hydrogen as a proton and subsequent enediol formation.

In an investigation of the mechanism of action of aldolase derived from rabbit muscle, it has been demonstrated that the alpha hydrogen of dihydroxyacetone phosphate is activated in the absence of any aldehyde acceptor.

Significance to NIAMD: The two enzymes studied mediate important steps in carbohydrate metabolism. It is felt that a more detailed understanding of these and other enzymatic reactions will aid in the approach to various metabolic diseases.

Proposed Course of Project: The stereospecificity of the isomerase with respect to the two H atoms on C-1 of fructose-6-phosphate will be studied. Aldolase will be further investigated in an attempt to learn whether the aldehyde moiety involved in the condensation can be activated by the enzyme in the absence of dihydroxyacetone phosphate.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD--35
SERIAL NUMBER

12. Research
BUDGET ACTIVITY

13. NO COOPERATING UNITS

14. NO PARALLEL RESEARCH IN PHS

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-35
SERIAL NUMBER

16. PUBLICATIONS FROM THIS PROJECT:

/Metabolism of Carbohydrates. A Review., D. Stetten, Jr., and Y. J. Topper, Am. J. of Med., 19, 96 (1955)./

17. NO HONORS OR AWARDS.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY

3. Intermediary Metabolism
SECTION

4. _____

5. NIAMD-36
SERIAL NO.

6. Study of Gluconic Acid Metabolism
PROJECT TITLE

7. Dr. I. G. Leder
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: The object of this project is to elucidate the mechanism by which gluconic acid is utilized in mammalian tissues.

Methods Employed: The enzyme which catalyzes the initial metabolic step in the utilization of gluconic acid, namely, the reaction gluconic acid $\longrightarrow$ gluconic acid-6-phosphate, is obtained in cell-free extracts and purified. By coupling this phosphorylation to gluconic acid-6-phosphate dehydrogenase and triphosphopyridine nucleotide the kinetics, stoichiometry and general properties of the kinase reaction are studied spectrophotometrically. Paper and column chromatography are used to identify reaction products.

Major Findings: The enzyme, gluconokinase, has been purified approximately 100 fold from hog kidney and the reaction product identified as gluconic acid-6-phosphate. A metal ion is required for enzymatic activity; this requirement may be met by Mg^{++}, Mn^{++}, Zn^{++}, Cc^{++}, or Ca^{++}. Gluconokinase appears to be specific for D-gluconate. Each of the following compounds tested was inactive: D-glucose, D-glycerate, D-glucuronate, D-altronate, D-galactonate, D-arabonate, L-arabonate, and L-gluconate.

Significance to NIAMD Research: Gluconic acid may be present in the human as a result of the limited action of liver glucose oxidase, or following the administration of gluconate as the calcium salt. The results of this study will reveal how this non-phosphorylated inter-

mediate is geared to the important glucose-6-phosphate oxidative pathway of carbohydrate metabolism and will thus further our understanding of normal and abnormal patterns of carbohydrate metabolism.

Proposed Course of Project: The study of the enzyme specificity will be extended to other sugar acids and to other nucleotide triphosphates. The stoichiometry and kinetics of this reaction as well as its possible occurrence in other tissues will be investigated.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-36
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. NO COOPERATING UNITS

14. NO PARALLEL RESEARCH IN PHS

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-36
SERIAL NO.

16. PUBLICATIONS FROM THIS PROJECT:

Crystalline Transketolase from Bakers' Yeat: Isolation and Properties. G. de la Haba, I. G. Leder and E. Racker, J. Biol. Chem., 214, 409 (1955).

17. NO HONORS OR AWARDS.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY

3. Intermediary Metabolism
SECTION

4. _____

5. NIAMD-37
SERIAL NO.

6. Humoral Antagonists of Insulin
PROJECT TITLE

7. Dr. James B. Field, Dr. DeWitt Stetten, Jr.
PRINCIPAL INVESTIGATORS

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: The objective of this project is to determine whether the sera of insulin-resistant diabetic patients contain an agent or agents which are responsible for the observed resistance. If so, a purification and characterization of the substance is envisioned.

Methods Employed: The physiological activity of insulin is determined by noting the augmentation of glycogenesis produced in the isolated rat hemidiaphragm in the presence of the hormone as compared to controls with no added insulin. Using data from such experiments as base-line, the sera obtained from suitable diabetic patients are assayed for anti-insulin activity. Physical methods of protein fractionation are used in attempts to concentrate the active material.

Patient Material:		No.	Average Stay in Days
Admissions:	Adult males	2	71
	Adult females	3	61
	Children male	0	
	Children female	0	
Outpatient:	No. of patients	1	
	No. of visits	2	

Major Findings: In those patients exhibiting an exalted insulin requirement during diabetic acidosis it has been possible to demonstrate insulin antagonism in the serum. Such activity is not related to the hormones of the adrenal cortex or the lowered pH of the serum, but seems

to be associated with a non-dialyzable component of the serum. Furthermore, the antagonist seems to migrate electrophoretically with the α and β globulins of the serum. It does not interfere with the binding of insulin to the rat hemidiaphragm. In one case the agent was no longer present 10 hours after the onset of therapy for acidosis.

Significance to NIAMD Research: The discovery of an insulin antagonist in the sera of patients with diabetic acidosis has given some insight into the increased insulin requirement exhibited by such patients. Further characterization of this material could give much needed information regarding the factors which determine insulin dosage in diabetes.

Proposed Course of Research: Further attempts will be made to more clearly define this antagonist and the mechanism of its production and action.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-37
SERIAL NUMBER

12. Research
BUDGET ACTIVITY

13. NO COOPERATING UNITS

14. Section on Endocrinology, National Institute of Arthritis and Metabolic Diseases
PARALLEL RESEARCH IN PHS

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-37
SERIAL NUMBER

16. PUBLICATIONS FROM THIS PROJECT:

Humoral Insulin Antagonism Associated with Diabetic Acidosis, J. B. Field and D. Stetten, Jr., Am. J. Med., (In press).

17. NO HONORS OR AWARDS.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY

3. Intermediary Metabolism
SECTION

4. _____

5. NIAMD-38
SERIAL NO.

6. The Metabolism of Glucuronic Acid
PROJECT TITLE

7. Dr. F. Eisenberg, Jr.; Dr. J. B. Field
PRINCIPAL INVESTIGATORS

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: The objective of this project is to elucidate the metabolic pathways of utilization of glucuronic acid in the mammal.

Methods Employed: Glucuronolactone, potassium glucuronate, and glucuronolactone-C^{14} were incubated with various tissues of the rat. End products of metabolism were detected and measured by chromatography, colorimetric analysis, and radioactive assay.

Glucuronolactone-6-C^{13} and sodium glucuronate-6-C^{13} were given by I.V. injection to normal men along with oral doses of menthol. Menthyl glucuronide was isolated from the urine and degraded. C^{13} abundance in the degradative fragments was determined by mass spectrometric analysis.

Major Findings: The principal immediate fate of glucuronolactone in the rat is hydrolysis to glucuronate. Of the tissues studied; liver, kidney, brain, spleen, testis, and diaphragm, only the liver has the capacity to carry out this reaction. When uniformly labeled glucuronolactone-C^{14} was incubated with either liver homogenate or slices no $C^{14}O_2$ was produced.

Glucuronolactone-6-C^{13} and sodium glucuronate-6-C^{13} were not incorporated into urinary menthyl glucuronide in man.

Significance to NIAMD: In view of the wide distribution of glucuronic acid in connective tissue, a defect in the metabolism of this

compound may be associated with arthritis and other diseases of that tissue. In order to recognize any derangement at the biochemical level in the diseased state it is essential to study the normal course of metabolism.

Proposed Course of Project: Further studies in the metabolism of glucuronic acid both *in vivo* and *in vitro* are planned.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-38
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. NO COOPERATING UNITS

14. NO PARALLEL RESEARCH IN PHS

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-38
SERIAL NO.

16. PUBLICATIONS FROM THIS PROJECT:

Studies on Glucuronide Conjugation in Man, F. Eisenberg, Jr., J. B. Field, and D. Stetten, Jr., Arch. Biochem. & Biophys., 59, 297 (1955).

17. NO HONORS OR AWARDS.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Biochemistry and Nutrition
LABORATORY

3. Intermediary Metabolism
SECTION

4. _____

5. NIAMD-39
SERIAL NO.

6. Metabolism of Glycogen
PROJECT TITLE

7. Dr. M. R. Stetten
PRINCIPAL INVESTIGATOR

8. Dr. D. Stetten, Jr.
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION

Objectives: To gain insight into the nature of the normal turnover of the glycogen of liver and muscle.

Methods Employed: Radioactive glucose is administered to animals and subsequently liver and muscle glycogen are isolated by extraction with acid or alkali. Glycogen is degraded enzymatically by various methods designed to split off glucose residues in a systematic fashion and to yield a series of limit dextrins. Glycogen samples are fractionated by means of centrifugation or alcohol precipitation and the isotope content and molecular weights determined. The method of light scattering is being used for the determination of molecular weights of the polysaccharide samples.

Major Findings: Glycogen has been found to be metabolically inhomogeneous in at least two parameters, within the individual molecules and within a given population of molecules. Turnover of glycogen molecules is not simply a replacement of pre-existing molecules by newly formed ones. Instead, glucose residues are added to, and removed from the non-reducing ends at the periphery of the polysaccharide molecules. Glucose residues find their way into the more centrally located layers by a process of branching. This branching process has been found to proceed much more rapidly with liver than with muscle glycogen. The correlation between iso-

tope content and molecular weight reveals that molecules of differing size differ in their metabolic activities. In the case of liver glycogen the smaller molecules are somewhat more reactive than the larger ones. In muscle the peripheral replacement of glucose residues of larger glycogen molecules is considerably more rapid than that of smaller molecules.

Significance to NIAMD Research: Alterations and defects in the way the body metabolizes various carbohydrates have been found to be characteristic of certain nutritional states, drug actions and metabolic diseases such as diabetes and Von Gierke's disease. Any additional knowledge as to how carbohydrates are normally handled may be expected to contribute to a better understanding of the nature of these conditions and diseases.

Proposed Course of Project: An explanation for the differences in turnover observed between muscle and liver glycogens is being sought. A comparative study will be made of the reactivity of glycogen molecules of different average molecular weights with phosphorylases from liver and from muscle.

The possibility that glycogen contains chemical linkages other than those generally recognized suggests itself from a number of observations made in the course of studies of glycogen isolated by different means. The possible occurrence and nature of such linkages is being studied.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-39
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. NO COOPERATING UNITS

14. NO PARALLEL RESEARCH IN PHS

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-39
SERIAL NO.

16. PUBLICATIONS FROM THIS PROJECT

Glycogen Regeneration in Vivo, M. R. Stetten and D. Stetten, Jr., J. Biol. Chem., 213, 723 (1955).

Glycogen Turnover, D. Stetten, Jr. and M. R. Stetten, (A chapter for a book honoring Dr. H. T. Clarke - in press.)

Disturbances in Intermediary Metabolism in Diabetes Mellitus, D. Stetten, Jr., Proc. Inst. Med. Chicago, 20, 284 (1955).

Biochemical Explorations of Certain Disturbances of Carbohydrate Metabolism, D. Stetten, Jr., Trans. & Studies of the Coll. of Phys., Phila., 22, 79 (1955).

Metabolic Relationship between Glutamic Acid, Proline, Hydroxyproline, and Ornithine, M. R. Stetten in "Amino Acid Metabolism", Johns Hopkins Press, Baltimore, 1955.

Metabolism of Glucose in Resting and Working Rats, D. J. Ingle, E. H. Morley and D. Stetten, Jr., Am. J. Physiol., 182, 263 (1955).

17. HONORS TO PERSONNEL RELATING TO THIS PROJECT

The Thirtieth Ludvig Hektoen Lecture of the Frank Billings Foundation of the Institute of Medicine of Chicago (D. Stetten, Jr.)

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD (INSTITUTE) 2. Chemistry (LABORATORY OR BRANCH)

3. Carbohydrates (SECTION) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NIAMD-40 (SERIAL NO.)

6. Labile Metabolites of Amino Acids
PROJECT TITLE

7. B. Witkop
PRINCIPAL INVESTIGATOR(S)

8. L. A. Cohen, A. A. Patchett, C. M. Foltz (Fellow)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Development of synthetic and analytical procedures to prove the presence of novel intermediates in the catabolism of amino acids which are too labile to be detected by customary routine methods.

Objectives: To show that a detailed knowledge of the oxidation mechanism of heterocyclic, aromatic and aliphatic model compounds makes possible the synthesis of missing or novel intermediates in the breakdown of physiologically important factors such as amino acids, ergot alkaloids, purines, vitamins etc. The availability of such intermediates obtained by synthesis makes it possible for the biochemist, pharmacologist and clinician to gain new insight into metabolic processes.

Methods Employed: New routes are being developed for the synthesis of compounds which so far have not been synthesized because the difficulties encountered have been too great or because the knowledge for the postulation of such compounds has been lacking. Special new techniques are employed in an attempt to simulate *in vitro* normal and abnormal oxidation processes occurring *in vivo*. Physico-chemical methods are being used and adapted to the study of the more subtle aspects of reactions and interactions of functional groups in model compounds as well as in higher molecular complexes. Additional cooperative projects with biochemical, pharmacological and clinical groups on the campus are being projected or have been started.

Major Findings: The important role of 5-hydroxytryptophan and its catabolites in the central nervous system prompted the synthesis of extremely labile 5,6-dihydroxyindole derivatives in collaboration with Dr. Arvid Ek, Dept. of Clinical Science and of Pathology, School of Medicine, University of Pittsburgh. The possible formation of tricyclic derivatives of β-substituted pseudo-tryptophans and -tryptamines to yield 2-carboxy-eseroline derivatives has been demonstrated. The new derivatives open a way to test some aspects of the biogenesis of eserine (physostigmine). The concept of the mechanism of oxidation

of indole compounds elaborated several years ago is being confirmed in a number of systems and is gaining importance, since more and more hydroxylated indoles are reported to have "psychogenic" properties. The metabolic fate of lysergic acid diethylamide ("LSD") which is being studied by Dr. J. Axelrod, NIMH, follows the patterns of previous oxidations carried out with simpler indoles. A detailed investigation on enamine-imine tautomerism in this connection led to new findings on the structure of dehydrobufotenine, a congener of the important base bufotenine and established further the mechanism of oxidation of cyclic and open (potential) azomethines and Schiff bases. Infrared spectrophotometry has been utilized to advantage in these studies. In collaboration with Dr. Th. Beiler, Stetson University, the preparation of pure di-p-iodobenzoyl derivatives of hydroxyamino acids (γ-hydroxyornithine) has been started, to be used in X-ray Fourier analysis. γ-Hydroxylysine, a regular constituent of collagen, has been converted chemically into the two diastereoisomers of a new amino acid, 5-hydroxypipecolic acid, subsequently found by Dr. P. Irreverre in dates, analyzed on Dowex-50 by K. Piez, prepared in quantity and synthesized by Dr. C. M. Foltz. New evidence completing the stereochemistry of the two forms of hydroxyproline, a major constituent of collagen, has been obtained by Dr. A. Patchett, who succeeded in preparing the long sought-for lactone of D- and L-allohydroxyproline, the keto forms of D- and L-hydroxyproline, the four crystalline N-carbobenzoxy derivatives in both series, and a number of other new derivatives to be tested for cytoactivity and in collagen-forming systems.

Dr. L. A. Cohen continued in the preparation and study of model compounds and succeeded in demonstrating the reality of peptide bond interactions through space. He is presently studying such effects in the natural cyclic polypeptide antibiotic gramicidin.

As a secondary problem new insights into the stereochemistry of ephedrine and pseudo-ephedrine have been gained by Dr. C. Foltz by converting the D-threo-1-phenyl-1,2-epoxypropane (from ephedrine) and the D-erythro-1-phenyl-1,2-epoxypropane (from pseudo-ephedrine) into the identical D-glycero-1-phenyl-2-hydroxypropane.

In extension of our previous collaboration with Dr. K. Piez, his method for the analysis of diastereoisomeric hydroxyamino acids was of great value in the analysis and separation of the hydroxyprolines, hydroxypipecolic acids and (in progress) γ-hydroxyglutamic acids.

Significance to Metabolic Diseases Research: Very recent biochemical and clinical investigations have led to surprising findings concerning the etiology of certain long-known diseases. Hereditary, nutritional or environmental factors produce deviations from standard metabolic patterns of amino acid degradation. In particular the following diseases have been associated with new or known amino acid metabolites: schizophrenia and other mental disorders, diabetes, and phenylpyruvic oligophrenia.

The new 5-hydroxytryptamine syndrome, the growing importance of serotonin in the central nervous system and in psychic disorders and the metabolic fate of LSD make imperative the preparation of many new hydroxyindole derivatives. Contributions along these lines are

9. PROJECT DESCRIPTION (Cont'd)

at present on a consultant level only but active participation is projected in collaboration with Drs. Brodie and Udenfriend in 1956.

Proposed Course of Project: In collaboration with Dr. Udenfriend a great many derivatives of the typical constituents of collagen will be tested in collagen-forming systems (tissue culture, if possible, and in burn blister areas) to gain insight into the biogenesis of this unique structural protein. The mechanism of formation of hydroxyproline will be studied in vitro and in vivo. The full stereochemistry, so far unknown, of δ-hydroxylysine and its cyclization product 5-hydroxypipecolic acid is being cleared up by applying a novel sequence of reactions elaborated in the hydroxyproline series. A fundamentally new concept is introduced into the study of enzyme mechanisms by the studies of Dr. Cohen on peptide interactions which is hoped to furnish insight into the helical and non-helical structure of proteins and the attachment of metabolites and substrates on enzyme surfaces, basic issues in our understanding of immunochemical reactions, transport mechanisms through membranes, carcinogenesis and genetic control of protein synthesis. An active program on cyclic peptides and chemical modifications relating to the insulin molecule will be started in February 1956.

In collaboration with Dr. J. Axelrod the structure of the metabolites from LSD and other ergot derivatives will be explored. Novel hydroxyindoles will be synthesized in collaboration with Dr. A. Ek, Dr. Brodie and Udenfriend and the structure of physiologically active indoxyl derivatives from enzymatic oxydation products of tryptophan and auxin will be investigated.

12. BUDGET ACTIVITY: Research

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956

Dr. S. Udenfriend (NHI)
Dr. K. Piez (NIDR)
Dr. A. Ek, Dept. of Clinical Science and Pathology, School of Medicine, University of Pittsburgh
Dr. P. Irreverre (LPB, NIAMD)
Dr. J. Axelrod (Clinical Investigations, NIMH)
Dr. Alton Meister (NCI, now Tufts Medical School, Brookline, Mass.)
Dr. T. Beiler, Stetson University

14. No parallel research in the Public Health Service.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

NIAMD-40

15. SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING 1955:

B. Witkop. Interconversions and Epimerizations of Open and Cyclic Hydroxyamino Acids. A Contribution to the Chemistry of the Building Stones of Collagen. London: The Chemical Society, 1955; Special Publication No. 3.

B. Witkop with R. K. Hill. The Synthesis of 2-Carboxydeoxyeserolines via β-Methyl-pseudo-tryptophan. J. Am. Chem. Soc., 77, 6592 (1955).

B. Witkop. Umwandlungen Offener und Cyclischer Oxyaminosäuren. Angewandte Chemie, 67, 765 (1955).

L. A. Cohen and B. Witkop. Transannular Reactions of Peptides. The Peptide Nitrogen in a 10-Membered Ring. J. Am. Chem. Soc., 77, 6595 (1955).

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING 1955:

Invitation by Chemical Society (London) to present lecture on current research (July 1955) to be published by the Society.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD — INSTITUTE
2. Chemistry — LABORATORY OR BRANCH
3. Carbohydrates — SECTION
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NIAMD-41 — SERIAL NO.
6. Microanalytical Laboratory — PROJECT TITLE
7. William C. Alford — PRINCIPAL INVESTIGATOR(S)
8. Paula M. Parisius, Evelyn G. Peake, Byron Baer, Mary Jean Barnett, guest (to August 1, 1955), Lillian J. Wyngarden (from October 17, 1955), William M. Jones, and Charles G. Remsburg — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION

The microanalytical laboratory is a service organization which provides necessary analytical services for research personnel of the National Institutes of Health. The scope of this work is summarized as follows:

1. About 10,000 elemental, functional group and instrumental analyses were performed during the past year. These, with the approximate number of each, include: carbon and hydrogen (2,500), nitrogen by Dumas, Kjeldahl and Nessler methods (2,300), reducing sugar (800), halogens (150), phosphorus (450), lactic acid (2,200 or 1,100 in duplicate), functional groups such as methoxyl, acetyl, carboxyl, active hydrogen (150), weight loss-moisture-ash etc. (450), metals such as sodium, potassium, iron, barium and antimony (50), miscellaneous (350), infra-red spectra (1,150), and optical rotations (150). Recipients of this service include approximately 100 research workers of the NIH staff. In addition, analyses are performed for governmental agencies outside the NIH, insofar as they can be handled without interfering with the progress of NIH research. Agencies which have received such service at various times include the Naval Medical Research Institute, Bethesda, Md., the Tuberculosis Research Laboratory, U.S.P.H.S., New York, the National Bureau of Standards, the Naval Research Laboratory, Anacostia, Maryland State Board of Health, Baltimore, and the Food and Drug Administration, Washington, D. C.

2. A laboratory operated by Mr. Jones provides infrared spectra for the Carbohydrate Section of the Laboratory of Chemistry and also for personnel of other Institutes insofar as his work load and schedule permit.

3. The services of Mr. Remsburg are available for various micro and macro analyses. For example, during the past year he has made over 2,000 lactic acid determinations and several hundred phosphorus

analyses for Dr. Du Buy of NMI. In addition he spent considerable time making complete analyses of crude sea salt mixtures which were being studied by Dr. O. Mickelson in regard to their dietary efficacy.

4. Consulting service is available to persons having special analytical problems. For example, a NCI chemist became involved in a controversy over the formula of a compound which he believed crystallized with two different solvents of crystallization, namely water and benzene. Special apparatus and technique were devised whereby the two solvents were isolated from the crystals, separated and identified so that no possible doubt remained as to their presence.

Significance to NIAMD and NIH: The work done by the microanalytical laboratory is of vital importance to many research scientists. The analyses aid in identifying unknown synthetic or natural products; provide proof of purity and identity of synthetic materials; and provide information needed to follow the progress, or direct the course, of various research programs. Satisfactory elemental analyses are required for publications describing the synthesis of new compounds.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-41
SERIAL NO.

11.

12. BUDGET ACTIVITY: Research

13. COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

The N.H.I. provided the salary of Mrs. M. J. Barnett (GS-9) for 7 months of the year after which this employee resigned.

14. No parallel research in the Public Health Service.

16. No publications.

17. No honors and awards.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD (INSTITUTES) 2. Chemistry (LABORATORY OR BRANCH)

3. Carbohydrates (SECTION) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NIAMD-42 (SERIAL NO.)

6. Chemistry of Stevioside and Related Substances
PROJECT TITLE

7. Hewitt G. Fletcher, Jr.
PRINCIPAL INVESTIGATOR(S)

8. Harry B. Wood, Jr. (Fellow to Oct. 4, Civil Service thereafter)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objective of this project is to elucidate the structure and configurations of the carbohydrate moieties of stevioside, the sweet, non-toxic principle of the Paraguayan plant Stevia Rebaudiana and to investigate the unique chemistry of some simpler analogs of stevioside.

Recent Progress: The work of the previous year (1954) had established the major features of the carbohydrate moieties in stevioside, including the remarkable fact that one glucose unit is present as the C_1 ester of a sterically hindered carboxyl group of the large aglucon. With alkali the glucose unit linked thus is removed as 1,6-anhydro-β-D-glucopyranose (levoglucosan). Since no C_1 esters of sterically hindered acids have hitherto been known, attention was turned during this past year (1955) to the synthesis of several simple examples. In place of the bulky and costly aglucon of stevioside, the readily available, sterically hindered acid 2,4,6-trimethylbenzoic acid (mesitoic acid) was chosen. The anomeric 2,3,4,6-tetra-O-acetyl-1-O-mesitoyl-D-glucopyranoses were synthesized and studied. When treated with alkali the β-anomer afforded (as had stevioside) levoglucosan; the α-anomer underwent complete destruction. On theoretical grounds this evidence indicates that the ester-linked sugar unit in stevioside is a β-D-glucopyranosyl moiety.

In the course of the above work the β-anomer was found to deacetylate in normal fashion when treated with cold methanolic hydrogen chloride. The α-anomer, on the other hand, deacetylated with this reagent but, here, the mesitoyl group was found to migrate simultaneously to C_2 as well. This surprising discovery, the migration of a sterically hindered acyl group from the α-C_1 to the C_2 position in glucopyranose, indicates that normal (non-hindered) acyl groups at the α-C_1 position must be exceedingly labile, if indeed they exist at all. The implications of this finding are too complicated to delineate here. It should, however, be pointed out that Dr. H. G. Khorana, in another laboratory, has almost simultaneously observed an analogous migration of a phosphate group in the D-ribofuranose series.

NIAMD-42
SERIAL NO.

Proposed Course of Project: The exact structure of the unusual disaccharide unit in stevioside will be investigated in the coming year.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-42
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. No cooperating units.

14. No parallel research in the Public Health Service.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING 1955:

H. B. Wood, Jr., R. Allerton, H. W. Diehl and H. G. Fletcher, Jr.
Stevioside I. The Structure of the Glucose Moieties.
J. Org. Chem., 20, 875 (1955).

H. G. Fletcher, Jr.
The Sweet Herb of Paraguay.
Chemurgic Digest, July-August, 1955.

H. B. Wood, Jr. and H. G. Fletcher, Jr.
Stevioside III. The Anomeric 2,3,4,6-Tetra-O-acetyl-1-O-mesitoyl-D-glucopyranoses and Their Behavior with Alkali.
J. Am. Chem. Soc., 78, in press.

H. B. Wood, Jr. and H. G. Fletcher, Jr.
A Migration of the Mesitoyl Group from C_1 to C_2 in α-D-Glucopyranose. Derivatives of 2-O-mesitoyl-D-glucose.
J. Am. Chem. Soc., 78, in press.

17. No honors and awards.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD (INSTITUTE) 2. Chemistry (LABORATORY OR BRANCH)

3. Carbohydrates (SECTION) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NIAMD-43 (SERIAL NO.)

6. Carbohydrates for Biochemical Research (formerly "Ribose Derivatives")
PROJECT TITLE

7. H. G. Fletcher, Jr.
PRINCIPAL INVESTIGATOR(S)

8. Robert K. Ness
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To extend knowledge of the chemistry of biochemically important sugars (such as ribose) with the objective of making these sugars and their derivatives available for biochemical investigations. One aspect of this project involves (a) publication of improved methods for the synthesis of sugar derivatives needed for biochemical research and (b) actual preparation of such substances for use by various groups, chiefly at NIH.

Recent Progress: (1) A study of the benzylideneation of D-ribose has led to a variety of crystalline products. One of these has been shown to be 2,3-O-benzylidene-D-ribofuranose which may prove useful in the synthesis of derivatives such as D-ribofuranose 1,6-diphosphate.

(2) The 1,2-O-(1-hydroxyalkylidene)-aldose structure has been postulated a number of times for certain highly reactive sugar esters and, at an earlier stage of this project, a labile, crystalline D-ribofuranose tribenzoate was considered to be 3,5-di-O-benzoyl-1,2-O-(1-hydroxybenzylidene)-α-D-ribofuranose. Attempts during the past year to synthesize 1,2-O-(1-hydroxybenzylidene)-α-D-ribopyranose and 2,3-(1-hydroxybenzylidene)-β-D-fructofuranose tribenzoate have failed although much novel chemistry was encountered in the process. This fact, together with the newly discovered C_1-C_2-migration of acyl groups (see project: "Chemistry of Stevioside and Related Substances") suggests that the substances assigned 1,2-O-(1-hydroxyalkylidene) structures are, in actual fact, 1-acyl sugars having the hydroxyl group at C_2 free. If this were true, a new path would be open for the synthesis of 2-substituted and 2-deoxy-sugars.

(3) As a byproduct of the work in (2) it was found that the benzyl group of 2,3-(1-benzyloxybenzylidene)-β-D-fructofuranose tribenzoate was readily replaced by an ethyl group through the action of very weakly acidic ethanol. This is a type of reaction which has not, hitherto, been observed.

(4) Numerous samples of sugars and sugar derivatives were supplied to various investigators at NIH and elsewhere, some, such as those for the diabetes research program, being exhaustively purified.

Proposed Course of Project: (1) The general problem of C_1 to C_2 migration of acyl groups will be pursued.

(2) Methods for the synthesis of biologically important carbohydrate derivatives will be studied.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-43
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. No cooperating units.

14. No parallel research in the Public Health Service.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING 1955:

H. G. Fletcher, Jr. and R. K. Ness.
Orthobenzoic Acid Derivatives of D-Ribopyranose. Preparation and Some Properties of 1,2-O-(1-Benzyloxybenzylidene)-α-D-ribopyranose and 1,2,4-O-orthobenzoyl-α-D-ribopyranose.
J. Am. Chem. Soc., 77, 5337 (1955).

R. K. Ness and H. G. Fletcher, Jr.
The Conversion of 1,4,6-Tri-O-benzoyl-2,3-O-(1-Benzyloxybenzylidene)-β-D-fructofuranose to 1,4,6-Tri-O-benzoyl-2,3-O-(1-ethoxybenzylidene)-β-D-fructofuranose by Acidic Ethanol.
J. Am. Chem. Soc., 78, in press.

17. No honors and awards.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD (INSTITUTE) 2. Chemistry (LABORATORY OR BRANCH)

3. Carbohydrates (SECTION) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NIAMD-44 (SERIAL NO.)

6. Higher-carbon sugars, anhydro sugars, sugar alcohols and their derivatives
PROJECT TITLE

7. Nelson K. Richtmyer
PRINCIPAL INVESTIGATOR(S)

8. James W. Pratt, Laura C. Stewart, Emmanuel Zissis
OTHER INVESTICATORS

9. PROJECT DESCRIPTION

Objectives: To evolve generalizations relating the physical and chemical properties of the groups of substances named in the project title to their configurations and conformations.

Recent Progress: In continuation of our studies on the formation of monomeric nonreducing anhydro sugars by the action of acids on hexoses, heptoses, and heptuloses, research in 1955 was focussed on the preparation of the desired sugars (D-allo-heptulose, D-altro-heptulose, L-galacto-heptulose, D-gluco-heptulose, D-manno-heptulose, D-talo-heptulose, 3-deoxy-D-arabo-hexose, and 3-deoxy-D-ribo-hexose), and the formation, isolation, characterization, and proof of structure of the anhydrides derivable from them. Especially significant were the preparation of the crystalline α-hexaacetate of D-altro-heptulose (sedoheptulose), proof of structure of the second sedoheptulosan as 2,7-anhydro-β-D-altro-heptulofuranose, isolation of what is presumably di-α-D-gluco-heptulopyranose 2,1':2',1-dianhydride, and the preparation and alkaline degradation of phenyl α-D-gluco-heptulopyranoside to 2,7-anhydro-β-D-gluco-heptulopyranose. The structure of the new tri-O-methylenevolemitol is under investigation.

Current and Projected Investigations: Continuation of these and closely related topics.

12. BUDGET ACTIVITY: Research

13. No cooperating units.

14. No parallel research in the Public Health Service.

Analysis of NIH Program Activities
Honors, Awards, and Publications Sheet

15. NIAMD-44
SERIAL NO.

16. PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING 1955:

Laura C. Stewart and Nelson K. Richtmyer.
Formation of 1,6- and 1,7-Anhydro-D-glycero-β-D-gulo-heptopyranoses from D-glycero-D-gulo-Heptose in Acid Solution.
J. Am. Chem. Soc., 77, 424-427 (1955).

Laura C. Stewart and Nelson K. Richtmyer.
Transformation of D-Gulose to 1,6-Anhydro-β-D-gulopyranose in Acid Solution.
J. Am. Chem. Soc., 77, 1021-1024 (1955).

James W. Pratt and Nelson K. Richtmyer.
Transformation of D-Allose to 1,6-Anhydro-β-D-allopyranose in Acid Solution.
J. Am. Chem. Soc., 77, 1906-1907 (1955).

Emmanuel Zissis and Nelson K. Richtmyer.
1,5-Anhydro-D-altritol.
J. Am. Chem. Soc., 77, 5154-5156 (1955).

Emmanuel Zissis and Nelson K. Richtmyer.
1,3-O-Benzylidene-2,5-di-O-p-tolylsulfonyl-DL-arabitol.
J. Am. Chem. Soc., 77, 5188-5189 (1955).

James W. Pratt and Nelson K. Richtmyer.
D-glycero-D-allo-Heptose, L-allo-Heptulose, D-talo-Heptulose and Related Substances Derived from the Addition of Cyanide to D-Allose.
J. Am. Chem. Soc., 77, 6326-6328 (1955).

Laura C. Stewart, Emmanuel Zissis and Nelson K. Richtmyer.
Die Bildung von 2.7-Anhydro-β-D-gluco-heptulopyranose durch Einwirkung von Säure auf D-Gluco-heptulose und von Alkali auf Phenyl-α-D-gluco-heptulopyranosid.
Chem. Ber. (in press).

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING 1955:

No honors and awards.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD (INSTITUTE) 2. Chemistry (LABORATORY)

3. Steroid (SECTION) 4. _____ (LOCATION) 5. NIAMD-45 (SERIAL NO.)

6. Analysis of Individual Corticoids in Urine. (PROJECT TITLE)

7. David F. Johnson (PRINCIPAL INVESTIGATOR)

8. Alma L. Hayden and Erich Heftmann (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objective: Improvement and refinement of present method.

Methods Employed: a) For isolation - extraction of urine at pH 1.0 and extraction after hydrolysis with β-glucuronidase (Glenn and Nelson, J. Clin. Endocrinol. and Metabolism, 13, 911 (1953)).

b) For fractionation - Heftmann and Johnson, Anal. Chem. 26, 519 (1954).

c) For determination - ultraviolet absorption and reduction of Blue Tetrazolium (Mader and Buck, Anal. Chem. 24, 666 (1952)).

d) For identification - paper chromatography, ultraviolet and infrared spectra and chemical tests on isolated fractions (with the co-operation of Dr. Erich Mosettig).

The method has been validated by recovery experiments and applied to the urines from normal men and women, pregnant women, and patients with rheumatoid arthritis (with the cooperation of Dr. J. J. Bunim), cirrhosis, adrenal hyperplasia, and other metabolic diseases.

Significance: Significant variations in the urinary excretion of individual adrenocortical hormones have been found, not only in the abnormal subjects but also in pregnancy and in normal men. A compound with an absorption maximum of 273 mμ has been isolated and structural studies of this material are under way. Changes in the excretion pattern of corticoids will be studied in normal pregnancy as well as pregnancy complicated by diabetes and toxemia (with the cooperation of the Diabetes Unit, Chronic Disease Branch, Bureau of State Services).

Analysis of NIH Program Activities

Budget Data, Honors, Awards and Publications Sheet

10. NIAMD-45
SERIAL NO.

12. BUDGET ACTIVITY:

RESEARCH [X]

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Dr. Mosettig and Dr. Bunim - for samples.

Dr. Mosettig for work on identification of isolated material.

14. NO PARALLEL RESEARCH IN THE PUBLIC HEALTH SERVICE.

16. NO PUBLICATIONS DURING CALENDAR YEAR 1955.

17. NO HONORS OR AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD (INSTITUTE) 2. Chemistry (LABORATORY)

3. Steroid (SECTION) 4. _____ (LOCATION) 5. NIAMD-46 (SERIAL NO.)

6. The Search for New Steroidal Sapogenins. (PROJECT TITLE)

7. Yoshio Sato (PRINCIPAL INVESTIGATOR)

8. H. George Latham, Jr. and Erich Mosettig (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objective: To isolate new steroidal sapogenins.

Methods Employed: The fruits of Solanum Panduraeforme E. Mey, an African plant, were found to yield principally solasodine, some solasodiene, lesser amounts of carpesterol and a very small amount of suspected solanocarpone. These compounds also occur in the fruits of Solanum Xanthocarpum, an Indian plant. The isolation of these substances are contrary to the findings of Veldsman and Louw [J. S. Afri. Chem. Inst., 2, 119 (1949)], who reported a new glyco alkaloid which yielded a new aglycone, similar in properties to solasonine and solasodine, as the sole constituent of the plant. We find no evidence of its presence.

The chemical behavior of the above isolated carpesterol indicates that it is not a sterol as was believed by the original Indian workers [Gupta and Dutt, J. Ind. Chem. Soc. XV, 95 (1938)] but a pentacyclic triterpene. Chemical studies are being continued on this interesting compound.

Significance to NIAMD:

A. Theoretical: Structural elucidation of terpenes.

B. Practical: Isolation of new compounds from natural sources may lead to substances of some pharmacological significance.

Analysis of NIH Program Activities

Budget Data, Honors, Awards and Publications Sheet

10. NIAMD-46
SERIAL NO.

12. BUDGET ACTIVITY:

RESEARCH [X]

13. NO COOPERATING UNIT.

14. NO PARALLEL RESEARCH IN THE PUBLIC HEALTH SERVICE.

16. NO PUBLICATIONS DURING CALENDAR YEAR 1955.

17. NO HONORS OR AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD (INSTITUTE) 2. Chemistry (LABORATORY)

3. Steroid (SECTION) 4. _____ (LOCATION) 5. NIAMD-47 (SERIAL NO.)

6. Stereochemistry of the Side Chain of Steroidal Alkaloids. (PROJECT TITLE)

7. Yoshio Sato (PRINCIPAL INVESTIGATOR)

8. H. George Latham, Jr. and Irving Scheer (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objective: The Structural Elucidation of Steroidal Alkaloids.

Methods Employed: The catalytic reduction of N-acetylated derivatives of tomatidine and solasodine leads to new dihydro derivatives resulting from the opening of the F ring of the molecule. The 26-N-ethyl derivatives (26-N-ethyltetrahydrosolasodine, 26-N-ethyldihydrotomatidine) of these dihydro compounds have been synthesized from the sapogenins, tigogenin acetate and neotigogenin acetate through a series of reactions. This confirms the existence of the C_{25} isomerism and the epimeric relationship of solasodine and tomatidine. Furthermore, it is another instance of the conversion of sapogenins into derivatives of steroidal alkaloids.

During the above synthesis it has been found that the alkaline treatment of 26-deoxy-26-iododihydrosapogenin acetates readily yields a common 26-deoxy-$\triangle^{25}$-dihydrosapogenin. This is the first instance of the conversion of the sapogenins, tigogenin and neotigogenin into a common unsaturated derivative and confirms the C_{25} epimeric relationship of these two sapogenins as in the manner shown previously for sarsasapogenin and smilagenin by Scheer, Kostic and Mosettig.

Analysis of NIH Program Activities

NIAMD-47
SERIAL NO.

Project Description Sheet

Significance:

A. Theoretical. The structural elucidation of these steroidal alkaloids and their interrelationship with the steroidal sapogenins is of classical fundamental importance in the field of steroid chemistry.

B. Practical. The study of the steroidal alkaloids may possibly be developed to the point of becoming a potential source for the production of steroidal hormones, including cortisone and its derivatives. These compounds may also be potential hypotensives, antifungal agents and bactericides.

Analysis of NIH Program Activities

Budget Data, Honors, Awards and Publications Sheet

10. NIAMD-47
SERIAL NO.

12. BUDGET ACTIVITY:

RESEARCH /X/

13. NO COOPERATING UNIT.

14. NO PARALLEL RESEARCH IN THE PUBLIC HEALTH SERVICE.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

"Synthesis and Stereochemistry of Some Solanidan-3-ones," by Y. Sato and H. George Latham, Jr., Chemistry and Industry, 444 (1955).

"Reversible Enzymatic Oxidation of Bile Acids," by O. Hayaishi,* Y. Sato, W. B. Jacoby and E. F. Stohlman, Arch. Biochem. and Biophys., 56, 554 (1955).

"Chemistry of Dihydrotomatidines," by Y. Sato and H. George Latham, Jr., J.A.C.S. (in press).

17. NO HONORS OR AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

* See project report of Dr. Hayaishi, as principal investigator.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD (INSTITUTE) 2. Chemistry (LABORATORY)

3. Steroid (SECTION) 4. _____ (LOCATION) 5. NIAMD-48 (SERIAL NO.)

6. Attempted Synthesis of Glucuronosides of Corticoids (PROJECT TITLE)

7. Edward M. Fry (PRINCIPAL INVESTIGATOR)

8. NO OTHER INVESTIGATORS.

9. PROJECT DESCRIPTION

Objective: The synthesis of tri-O-acetyl-β-D-glucopyranurono-6,1-lactone was achieved but this compound proved valueless as an intermediate in the synthesis of glucuronosides. While a preliminary attempt to make a glucuronoside of desoxycorticosterone by the classical method was in progress, the papers by Isselbacher and Axelrod (JACS 1955) and by Schneider, Lieberman, et al., (JACS 1955) showed that glucuronoside formation in tetrahydrocortisone takes place at position 3, and conjugation at C-21 is biologically insignificant.

The question whether the hydroxyl group of the C-17 ketol side chain is capable of conjugation still remains open, and experiments to settle this point will be resumed.

Significance: The practical and theoretical significance of these studies lies in their application to corticoid analysis in urine and blood and the interpretation of corticoid metabolism.

Analysis of NIH Program Activities

Budget Data, Honors, Awards and Publications Sheet

10. NIAMD-48
SERIAL NO.

12. BUDGET ACTIVITY:

RESEARCH [X]

13. NO COOPERATING UNIT.

14. NO PARALLEL RESEARCH IN THE PUBLIC HEALTH SERVICE.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

"Tri-O-acetyl-β-D-glucopyranurono-6,1-lactone," by Edward M. Fry, J.A.C.S., 77, 3915 (1955).

17. NO HONORS OR AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD (INSTITUTE) 2. Chemistry (LABORATORY)

3. Steroid (SECTION) 4. _____ (LOCATION) 5. NIAMD-49 (SERIAL NO.)

6. a. Preparation of Several New Anthrasteroids and Observations on the Dehydrobromination of 7-Bromo-Δ^5-steroids.

b. Elucidation of the Pathway in the Conversion of Dehydroergosterol to Anthraergostapentaene.
(PROJECT TITLE)

7. William R. Nes and Erich Mosettig
(PRINCIPAL INVESTIGATORS)

8. Robert B. Kostic
(OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

This work comprises the completion of projects a. and b. (Nes, Mosettig, Kostic) reported December 1954.

a. Objective: The extension of the anthrasteroid rearrangement to a wider range of steroids including those occurring in the animal organism.

Methods Employed: Anthracholestatetraene, anthracholestatriene, methyl anthrabisnorcholatetraenate, and methyl anthrabisnorcholatrienate have been prepared from the corresponding steroidal 5,7,9(11)-trienes derived from cholesterol and 3-hydroxycholenic acid, respectively. The molecular rotations of these anthrasteroids and those of the corresponding derivatives in the ergosterol series exhibit consistent ΔM_D values for hydrogenation of the conjugated double bond. This is interpreted as evidence for a common position of the conjugated double bond in the various anthrasteroids and, consequently, for a dominant pathway for the over-all conversion. Rearrangement in the 17-ketosteroid series (epiandrosterone) gave in poor yield the corresponding chloroanthrastatrienone. This compound exhibited the normal ultraviolet spectrum of anthrastatrienes and possessed an infrared spectrum which showed the presence of an unconjugated carbonyl group. This is considered as corroborating earlier evidence that anthrasteroids possess an aromatic B-ring and not an aromatic C-ring. A study of the influence of temperature on the dehydrobromination of 7-bromocholesteryl acetate

revealed that the yield of $\Delta^{5,7}$-steroid decreases with decreasing temperature while the formation of the 4,6-isomer was not appreciably affected. The isocaproates of cholesterol and dehydroepiandrosterone gave better yields of the corresponding $\Delta^{5,7}$-steroid than did the acetates.

b. Objective: Elucidation of the pathway of anthrasteroid rearrangement.

Methods Employed: An intermediate hydrocarbon which is considered to be 5,7,9(11),14,22-ergostapentaene has been isolated from the reaction mixture when dehydroergosterol was treated with hydrogen chloride in chloroform. The hydrocarbon was convertible to anthraergostapentaene in 86% yield under the same conditions. That dehydration of dehydroergosterol precedes rearrangement and aromatization in the course of its conversion to anthraergostapentaene was also inferred from kinetic data as well as from the behavior of model compounds.

3,5-Cyclo-6,8(14),9(11),22-ergostatetraene has been prepared and shown not to participate in the acid-catalyzed reaction of dehydroergosterol. On treatment of the i-steroid with hydrogen chloride an addition reaction took place yielding dehydroergosteryl chloride.

Significance to a. and b.: The significance of the above work lies in the obvious speculation that the "anthrasteroid rearrangement" may be part of a metabolic pathway to spontaneous carcinogenesis. Moreover, the pathway of this unusual rearrangement has been established by the isolation and characterization of an important intermediate [5,7,9(11),-14,22-ergostapentaene].

Analysis of NIH Program Activities

Budget Data, Honors, Awards and Publications Sheet

10. NIAMD-49
SERIAL NO.

12. BUDGET ACTIVITY:

RESEARCH /X/

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Dr. Murray J. Shear, Laboratory of Chemical Pharmacology, National Cancer Institute.

14. NO PARALLEL RESEARCH IN THE PUBLIC HEALTH SERVICE.

16. NO PUBLICATIONS DURING CALENDAR YEAR 1955.

17. NO HONORS OR AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD (INSTITUTE) 2. Chemistry (LABORATORY)

3. Steroid (SECTION) 4. _____ (LOCATION) 5. NIAMD-50 (SERIAL NO.)

6. Reduction Products of Some Sterol Carboxylic Acids; Polyamines and Quarternary Ammonium Compounds Derived from Sterols.
PROJECT TITLE

7. Prof. Hans Lettré and William R. Nes
PRINCIPAL INVESTIGATORS

8. Ch. Scholtissek and H. Endo (both of the Cancer Research Institute at Heidelberg)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: An approach to the study of the biogenesis of steroids in the tumor cell.

Methods Employed: These studies comprise the chemical synthesis of 2,3-secocholestane-2,3-diacid and a number of its analogs and their conversion to the corresponding di- and monoalcohols, -aldehydes, -amines and -quarternary salts. These compounds are steroids in which the ring system has been opened between positions 2 and 3 and in which the various functional groups mentioned have been introduced on the terminal carbon atoms. Analogous compounds were prepared with ring-opening between positions 3 and 4 and with functional groups in other positions. A study is being made as to whether these compounds can be used by tumor or normal cells for the synthesis of cholesterol and also as to whether certain of these degradation products might be antimetabolites causing the inhibition of cholesterol genesis and consequent inhibition of tumor growth. The mouse Ehrlich ascites tumor is being used and Dr. Nes has had the opportunity of learning the technique and of aiding to some extent in the pharmacologic examination. A study is also being made of the effect of these compounds in tissue cultures.

Significance: The comparative study of cholesterol biogenesis in the normal and the tumor cell; the search for cancer antimetabolites desired from cholesterol.

Analogous degradative and partial-synthetic procedures will be applied to tagged cholesterol (C^{14}-4) which should make it possible to observe the metabolism of the above-mentioned sterol derivatives in vivo.

Analysis of NIH Program Activities

Budget Data, Honors, Awards and Publications Sheet

10. NIAMD-50
SERIAL NO.

12. BUDGET ACTIVITY:

RESEARCH /X/

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Institute of Experimental Cancer Research, University of Heidelberg, Germany.

14. NO PARALLEL RESEARCH IN THE PUBLIC HEALTH SERVICE.

16. NO PUBLICATIONS DURING CALENDAR YEAR 1955.

17. NO HONORS OR AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD (INSTITUTE) 2. Chemistry (LABORATORY)

3. Steroid (SECTION) 4. _____ (LOCATION) 5. NIAMD-51 (SERIAL NO.)

6. Elucidation of the Stereochemistry of the Spiroketal Side Chain of Steroidal Sapogenins.
PROJECT TITLE

7. Irving Scheer and Erich Mosettig
PRINCIPAL INVESTIGATORS

8. Malcolm Thompson
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objective: Determination of the configuration of carbon C-22 in the sapogenin side chain.

Methods Employed: In continuation of the program to determine whether the sapogenins exhibit isomerism at C-22 three approaches were made: First, in a joint project with the Eastern Research Laboratory of the Department of Agriculture, it was determined that the existence of the reported lay sapogenins, the cholegenins, was highly doubtful (in ox-bile from American sources, at any rate). Second, a program was started to eliminate the asymmetric center at C-25 in sarsasapogenin and smilagenin by way of the corresponding C-23 bromo compounds. Third, the sapogenin precursor, kryptogenin, was extensively studied in reduction reactions. From the latter study two new compounds believed to be 22,26-epoxy isomers were isolated and a start made on the proof of their structure. From kryptogenin a method was developed for the preparation of 26-hydroxycholesterol, a compound which appears to be of interest in metabolic studies. (Dr. D. S. Frederickson, Heart Institute, Laboratory of Cellular Physiology and Metabolism.)

Significance: a) To settle a widely disputed point in the stereochemistry of the sapogenin side chain. b) To interpret the biological hydroxylation of the appropriate sterols in the plant and animal organism.

Analysis of NIH Program Activities

Budget Data, Honors, Awards and Publications Sheet

10. NIAMD-51
SERIAL NO.

12. BUDGET ACTIVITY:

RESEARCH [X]

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Eastern Utilization Research Branch, U. S. Department of Agriculture, Philadelphia, Pennsylvania

14. NO PARALLEL RESEARCH IN THE PUBLIC HEALTH SERVICE.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

"The C-25 Isomerism of Smilagenin and Sarsasapogenin," by Irving Scheer, Robert B. Kostic and Erich Mosettig, J.A.C.S., 77, 641 (1955).

"Coprostane, Cholestane and their 16β-Hydroxy Derivatives from Steroidal Sapogenins," by Irving Scheer and Erich Mosettig, J.A.C.S., 77, 1820 (1955).

17. NO HONORS OR AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD (INSTITUTE) 2. Chemistry (LABORATORY)

3. Steroid (SECTION) 4. _____ (LOCATION) 5. NIAMD-52 (SERIAL NO.)

6. The Infrared Spectral Characteristics of the Morphine Alkaloids. (PROJECT TITLE)

7. Irving Scheer and Harold K. Miller (PRINCIPAL INVESTIGATORS)

8. Theodore D. Perrine, Lyndon F. Small and Lewis J. Sargent (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objective: A catalogue of infrared spectra of the morphine alkaloids, their degradation products, simpler analogues and related synthetic compounds has been prepared.

Significance: This is to serve as a characterization of functional groups and steric arrangements of groups and rings.

Analysis of NIH Program Activities

Budget Data, Honors, Awards and Publications Sheet

10. NIAMD-52
SERIAL NO.

12. BUDGET ACTIVITY:

RESEARCH /X/

13. NO COOPERATING UNIT.

14. NO PARALLEL RESEARCH IN THE PUBLIC HEALTH SERVICE.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955.

"The Spectral Characterization of Benzenoid Systems Derivable from $\Delta^{5,7}$-Steroids," by Irving Scheer, William R. Nes and Phyllis B. Smeltzer, J.A.C.S., 77, 3300 (1955).

17. NO HONORS OR AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD (INSTITUTE) 2. Chemistry (LABORATORY)

3. Steroid (SECTION) 4. _____ (LOCATION) 5. NIAMD-53 (SERIAL NO.)

6. Study of Fecal Steroids. (PROJECT TITLE)

7. Erich Heftmann (PRINCIPAL INVESTIGATOR)

8. Alma L. Hayden and Erich Mosettig (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION:

Objective: Complete analysis of the steroidal content of the feces.

Methods Employed: Fifty kg. of stool from healthy men was collected in alcohol and, with the cooperation of the Section on Fractionation and Isolation (Dr. J. C. Keresztesy) extracted with alcohol. The extract was separated into a neutral and acidic fraction (bile acids, estrogens, etc.). The neutral fraction was partitioned between 80% alcohol and petroleum ether to remove material of low polarity (sterols, etc.) and then separated into ketonic and non-ketonic material by Girard separation. The ketonic fraction was subjected to repeated partition chromatography. The ketonic substances were on the chromatograms and were then eluted. We now have about 60 fractions, seven of which are in a relatively high state of purity and give the Zimmerman test for ketosteroids.

Significance: It is planned to isolate and characterize in succession the ketonic, non-ketonic, acidic, and relatively non-polar lipides in the stool sample. The steroid composition of stool from normal men is a basic piece of information needed for further work on the metabolism of steroids in man.

Analysis of NIH Program Activities

Budget Data, Honors, Awards and Publications Sheet

10. NIAMD-53
SERIAL NO.

12. BUDGET ACTIVITY:

RESEARCH /X/

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Section on Fractionation and Isolation, Laboratory of Biochemistry and Nutrition, NIAMD - for extraction and concentration of extracts.

14. NO PARALLEL RESEARCH IN THE PUBLIC HEALTH SERVICE.

16. NO PUBLICATIONS DURING CALENDAR YEAR 1955.

17. NO HONORS OR AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD (INSTITUTE) 2. Chemistry (LABORATORY)

3. Steroid (SECTION) 4. _____ (LOCATION) 5. NIAMD-51 (SERIAL NO.)

6. Infrared Spectra of Adrenocortical Hormones. (PROJECT TITLE)

7. Alma L. Hayden (PRINCIPAL INVESTIGATOR)

8. Erich Heftmann (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Methods Employed: The infrared spectra of six adrenocortical hormones were determined in potassium bromide disks, using the Perkin-Elmer double-beam infrared spectrometer.

Significance to NIAMD Research: It is now possible to identify as little as 300 micrograms of material in the solid state. The poor solubility of adrenocortical steroids in infrared transparent solvents has made it impossible to obtain solution spectra of the unesterified hormones. The method is of great value in the identification of small quantities of adrenocortical hormones isolated from natural sources.

Analysis of NIH Program Activities

Budget Data, Honors, Awards and Publications Sheet

10. NIAMD-54
SERIAL NO.

12. BUDGET ACTIVITY:

RESEARCH /X/

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

H. K. Miller, National Institute of Arthritis and Metabolic Diseases, Laboratory of Chemistry - infrared analyses and design of die and disk holder.

Instrument Section, National Institutes of Health - construction of equipment.

14. NO PARALLEL RESEARCH IN THE PUBLIC HEALTH SERVICE.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

"Partition Chromatography of Steroids," by Erich Heftmann, Chem. Reviews, 55, 679 (1955).

"Infrared Spectra of Adrenocortical Hormones in Potassium Bromide Disks," by Alma L. Hayden, Anal. Chem., 27, 1486 (1955).

17. NO HONORS OR AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD (INSTITUTE) 2. Chemistry (LABORATORY)

3. Steroid (SECTION) 4. _____ (LOCATION) 5. NIAMD-55 (SERIAL NO.)

6. The Structure of the Aglycone of Stevioside. (PROJECT TITLE)

7. Fred Dolder and Erich Mosettig (PRINCIPAL INVESTIGATORS)

8. William R. Nes (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION: This is a continuation of the project given under the same heading in the report of December 1954.

Objective: To determine the fine structure of the aglycone.

Methods Employed: In regard to the structure of "steviol" and "isosteviol" there remain to be definitely established the positional and steric location of the carboxylic group and methyl groups, respectively, and the steric juncture of the four hydroaromatic rings. Appropriate chemical reactions as well as physical measurements are being employed for establishing these finer structural points.

Significance: The steviol-isosteviol structure has become of very acute interest since it could be tied up with the garrya alkaloids and more recently, also with cafestol (extracted from coffee beans).

On account of the high sweetening power of the parent glycoside stevioside interest was expressed by some industrial concerns, and long-range toxicity studies appeared desirable. The major objection to pursuing these practical problems is the rarity of the source-material, which apparently can be secured only in Paraguay.

Analysis of NIH Program Activities

Budget Data, Honors, Awards and Publications Sheet

10. NIAMD-55
SERIAL NO.

12. BUDGET ACTIVITY:

RESEARCH [X]

13. NO COOPERATING UNIT.

14. NO PARALLEL RESEARCH IN THE PUBLIC HEALTH SERVICE.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

"Stevioside. II. The Structure of the Aglucon," by Erich Mosettig and William R. Nes, J. Org. Chem., 20, 884 (1955).

17. NO HONORS OR AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD (INSTITUTE) 2. Chemistry (LABORATORY)

3. Steroid (SECTION) 4. _____ (LOCATION) 5. NIAMD-56 (SERIAL NO.)

6. Synthesis of Isomers of Cocaine. (PROJECT TITLE)

7. Stephen P. Findlay (PRINCIPAL INVESTIGATOR)

8. NO OTHER INVESTIGATORS.

9. PROJECT DESCRIPTION:

There are theoretically possible four isomers of cocaine, of which only two are known. There is no way to predict the physiological action of the missing forms. The synthesis of 2-carbomethoxytropinone has been perfected. This substance is an essential intermediate in the synthesis of the desired allococaine and allopseudococaine. This work has involved a fundamental study of the mechanism of conversion of pyrrols to α,δ-alkane dioximes. The latter are key substances for preparation of the α,δ-alkane diamines, the end products of many physiological processes. The dioximes also provide the only practical source of the dialdehydes which are essential to the total synthesis of the cocaine isomers.

Analysis of NIH Program Activities

Budget Data, Honors, Awards and Publications Sheet

10. NIAMD-56
SERIAL NO.

12. BUDGET ACTIVITY:

RESEARCH /X/

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

U. S. Bureau of Narcotics, Treasury Department.

14. NO PARALLEL RESEARCH IN THE PUBLIC HEALTH SERVICE.

16. NO PUBLICATIONS DURING CALENDAR YEAR 1955.

17. NO HONORS OR AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD (INSTITUTE) 2. Chemistry (LABORATORY OR BRANCH)

3. Analgesics (SECTION) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NIAMD-57 (SERIAL NO.)

6. Hydroxycodeine narcotics
PROJECT TITLE

7. Lyndon F. Small
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

By reaction of thebaine with methyl hypobromite followed by sodium acetate and hydrolysis, a new series of hydroxycodeinone derivatives has been obtained. These are isomeric with the known powerful narcotics Eukodal and Numorphan. Structural investigation and pharmacological evaluation are not yet complete.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-57
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956

Merck & Co., Rahway, N. J., and Mallinckrodt Chemical Wks., St. Louis, Mo.

14. No parallel research in the Public Health Service.

16. No publications.

17. No honors and awards.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD — INSTITUTE
2. CHEMISTRY — LABORATORY OR BRANCH
3. Analgesics — SECTION
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NIAMD-58 — SERIAL NO.
6. Antagonists to barbiturates — PROJECT TITLE
7. Lyndon F. Small — PRINCIPAL INVESTIGATOR(S)
8. T. D. Perrine — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION

There is urgent need for an effective and safe agent against accidental, suicidal or idiosyncratic barbiturate poisoning. Some recent work suggests that derivatives of glutarimide, remotely resembling barbiturates in structure, may be effective. A series of glutarimide derivatives and allied compounds has been prepared for testing in this field, no results of tests yet available.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-58
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S)(ITEM 10)

Pharmacological testing by Dr. Franklin Snyder, Harvard Medical School and Boston Lying-in Hospital.

Lilly Research Laboratories has supplied some spirobarbituric acid compounds for this project.

14. No parallel research in the Public Health Service.

16. No publications.

17. No honors and awards.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD — INSTITUTE
2. Chemistry — LABORATORY OR BRANCH
3. Analgesics — SECTION
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NIAMD-59 — SERIAL NO.

6. Reduction products from thebaine
PROJECT TITLE

7. Lyndon F. Small
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Minor by-products formed in the reduction of thebaine, important for an understanding of reduction mechanism, have never been adequately investigated. Residues from reduction of 190 lb. of thebaine were worked up for this purpose. After removal of hitherto-known constituents, about 1% of the new alkaloid neopine methyl ether was isolated and its structure proved. This demonstrates that the reduction proceeds, in part at least, in an entirely unsuspected manner. Project completed.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-59
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR ANY OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956.

Reduction residues were furnished by Merck & Co., Rahway, N. J.

14. No parallel research in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

The Reduction of Thebaine. Neopine Methyl Ether.
Lyndon F. Small
Journal of Organic Chemistry, 20, 953-958 (1955).

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases

2. Laboratory of Chemistry 3. Section on Analgesics

4. _____ 5. SERIAL NO. NIAMD-60

6. Synthesis of Dihydrothebaine Degradation Products.

7. Lewis J. Sargent 8. None

9. PROJECT DESCRIPTION:

OBJECTIVES:

To elucidate the structure of the analgesic drug - metopon.

METHODS:

Metopon was degraded to a tetrahydronaphthalene derivative in which the only uncertainty of structure is the location of the methyl group which constitutes the unique feature of metopon. Because of steric difficulties inherent in the synthesis of such a highly substituted tetrahydronaphthalene system, several logical approaches have failed. An equally valid proof will be found in the synthesis of the corresponding dialkyl amino-octahydrophenanthrene system. Several of these types have been prepared and tested for analgesic activity, which was of such low order as not to offer any promise of practical value.

10. SERIAL NO. NIAMD-60

12. BUDGET ACTIVITY: Research

13. No report.

14. No parallel research in the Public Health Service.

16. No publications

17. No honors or awards.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases

2. Laboratory of Chemistry 3. Section on Analgesics

4. _____ 5. SERIAL NO. NIAMD-61

6. PROJECT TITLE: Synthesis of New Compounds which may have Analgesic Effect.

7. Theodore D. Perrine 8. None

9. PROJECT DESCRIPTION:

OBJECTIVES:

The synthesis of new chemical types or modification of compounds with known analgesic action to obtain new and improved drugs for relief of pain and to augment data on relation of structure to activity.

METHODS AND RESULTS:

1. Work has continued on the attempt to enlarge the nitrogen ring in dihydrocodeine. This synthesis appears now to be successful but must be repeated on a larger scale for confirmation and testing of activity. This work was greatly facilitated by a gift of 10-hydroxydihydrocodeine from Mallinckrodt Chemical Works, obviating the preparation of this starting material.

2. The pyridine-CrO_3 oxidation of acetyldihydrocodeine to N-formylnordihydrocodeine was accomplished.

3. Work on compounds containing a quaternary carbon has continued, being directed particularly toward the preparation of 4-phenylquinuclidine. 4-Vinyl-4-phenylpiperidine has been prepared and an effort will be made to cyclize this to the quinuclidine.

4. A new method of synthesis resulting in addition of phenethyl-like substituents to the heterocyclic nitrogen of meperidine resulted in the preparation of three new compounds of this type: 4-carbethoxy-4-phenyl-1-(2-phenylethyl)-piperidine; 4-carbethoxy-4-phenyl-1-(2-phenyl-2-hydroxyethyl)-piperidine; and 4-carbethoxy-4-phenyl-1-(2-phenyl-2-acetoxyethyl)-piperidine. The first two have about three times the analgesic

potency of meperidine. They are being tested for addiction liability. Acetylation of the hydroxyl (third compound) practically destroyed analgesic effectiveness.

5. Accompanying the synthetic work a significant amount of time and effort has gone into improvement in technical procedure and devices to that end (see award citation under item 17).

10. SERIAL NO. NIAMD-61

12. BUDGET ACTIVITY: Research

13. Complementary to pharmacological investigation in this section, Serial No. NIAMD-66

14. None

16. Perrine, T. D. "Needle-valve stopcock". Analyt. Chem. In press.

Perrine, T. D. and Eddy, N. B. "Preparation and Analgesic Activity of 4-carbethoxy-4-phenyl-1-(2-phenylethyl)-piperidine and related compound". J. Org. Chem. In press.

Perrine, T. D. and Small, L. F., "Oxidation of acetyldihydrocodeine". J. Org. Chem. In press.

17. Won an "Awards" citation for invention of a stopcock with needle valve control.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases

2. Laboratory of Chemistry 3. Section on Analgesics

4. _____ 5. SERIAL NO. NIAMD-62

6. PROJECT TITLE "Synthesis of Structures Related to Morphine".

7. James G. Murphy 8. None

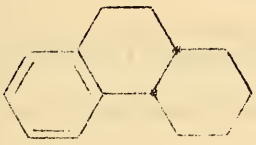
9. PROJECT DESCRIPTION:

OBJECTIVES:

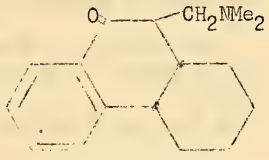
5. Synthesis of amino-octahydrophenanthrenes. As part of the program of synthesis of new and improved drugs for the relief of pain and of compounds designed to help correlate structure and activity, this work is a continuation of the investigation of the ring system cis-as-octahydrophenanthrene I. This structure replicates the hydrogenated phenanthrene system of morphine both in regard to the disposition of the aromatic nucleus and the mode of fusion of the two alicyclic rings.

METHODS AND RESULTS:

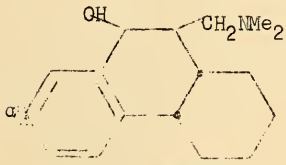
cis-1,2,3,4,4a,10a-Hexahydro-10-dimethylaminomethyl-9(10H)-phenanthrone, II, cis-1,2,3,4,4a,9,10,10a-octahydro-10-dimethylaminomethyl-9-phenanthrol, III, and cis-1,2,3,4,4a,9,10,10a-octahydro-9-acetoxy-10-dimethylaminomethylphenanthrene, IV, of this series have been previously reported.



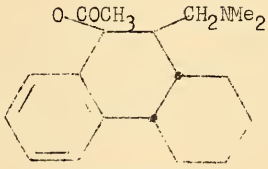
I



II (NIH #7258)

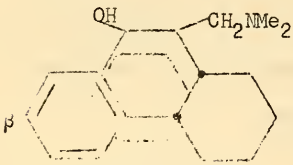


III (NIH #7262)

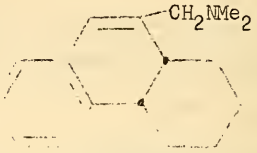


IV (NIH #7265)

The principal product from the lithium aluminum hydride reduction of II is III. It has now been found that the epimeric alcohol is also produced in this reduction but in minor yield. Accordingly, III is given the alpha designation. The β-epimer, V, is produced in high yield by the buffered platinum reduction of II.



V (NIH #7316)



VI (NIH #7329)

Both alpha and beta phenanthrols (III and V), on treatment with concentrated hydrochloric acid at room temperature, undergo dehydration in high yield to cis-1,2,3,4,4a,10a-hexahydro-10-dimethylaminomethylphenanthrene, VI.

Catalytic reduction of VI gave cis-1,2,3,4,4a,9,10,10a-octahydro-10-dimethylaminomethylphenanthrene, VII.



VII

It was observed that the d-10-camphorsulfonate salt of II had unusual lipid solubility. A sample of this was therefore prepared for screening (NIH #7330).

The series has shown low to moderate analgesic activity. Of the group, II shows the highest activity (27 mg./kg. subcut., mice). [illegible] oral ED_{50} of 59 mg./kg. (mice) equals meperidine in this respect.

10. SERIAL NO. NIAMD-62

12. BUDGET ACTIVITY: Research

13. Complementary to pharmacological investigations in this section, Serial No. NIAMD-66

14. None

16. May, E. L. and Murphy, J. G. "Structures Related to Morphine. III. Synthesis of an Analog of N-methylmorphinan", J. Org. Chem., 20, 257-263, 1955.

May, E. L. and Murphy, J. G. "Structures Related to Morphine. IV. m-Substituted Phenylcyclohexane Derivatives", J. Org. Chem., 20, 1197-1201, 1955.

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases

2. Laboratory of Chemistry 3. Section on Analgesics

4. _____ 5. SERIAL NO. NIAMD-63

6. PROJECT TITLE: Synthesis of structures related to morphine.

7. Everette L. May 8. James G. Murphy

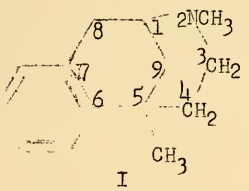
9. PROJECT DESCRIPTION:

OBJECTIVES:

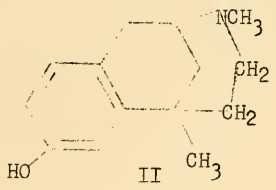
Synthesis of new and improved drugs for the relief of pain and of compounds designated to help correlate structure with activity and deleterious side effects.

METHODS AND RESULTS:

4. Synthesis of derivatives of 2,5-(dimethyl)-6,7-benzmorphan. The report for 1954 described parent compound I, which was half as active analgesically as meperidine and less toxic. Nitration of I followed by reduction and diazotization converted I in low yields into two isomeric hydroxybenzmorphans. One of these isomers was at least twice as active an analgesic as I and was believed, therefore, to be the m-hydroxy compound (II). Further study of analgesic and toxic effects as well as proof of structure of II, are awaiting its total synthesis, now underway.



I



II

The synthesis of other analogs of I and II are in progress, analogs which would place an additional methyl group at position 9. Such an addition would put these compounds closer to N-methylmorphinan and 3-hydroxy-N-methylmorphinan stereochemically.

10. SERIAL NO. NIAMD-63

12. BUDGET ACTIVITY: Research

13. Complementary to pharmacological investigations in this section, Serial No. NIAMD-66

14. None

15. None

16. May, E. L. and Murphy, J. G. "Structures Related to Morphine. III. Synthesis of an Analog of N-methylmorphinan", J. Org. Chem., 20, 257, 1955.

May, E. L. and Murphy, J. G. "Structures Related to Morphine. IV. m-Substituted Phenylcyclohexane Derivatives", J. Org. Chem., 20, 1197, 1955.

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases

2. Laboratory of Chemistry 3. Section on Analgesics

4. _____ 5. SERIAL NO. NIAMD-64

6. PROJECT TITLE: Synthesis of Structures Related to Morphine.

7. Everette L. May 8. None

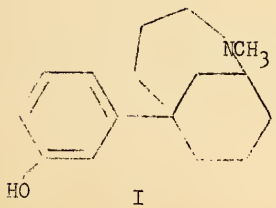
9. PROJECT DESCRIPTION:

OBJECTIVES:

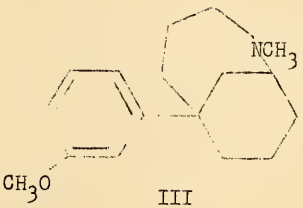
Synthesis of new and improved drugs for the relief of pain and of compounds designed to help correlate structure with activity and deleterious side effects. Also to develop and improve chemical methods and techniques principally from the point of view of synthesis.

METHODS AND RESULTS:

1. Synthesis of azabicyclodecanes derived from 2-(m-methoxyphenyl)-cyclohexanone. The synthesis of these compounds was undertaken because the desired product (I) is a homolog of a highly active analgesic, 5-(m-hydroxyphenyl)-2-methylmorphan (II), (described in 1954 report), a homolog which could be expected to be of lower addiction liability. However, the azabicyclo (4.3.1) decane ring structure proved surprisingly labile so that the yields accompanying the synthesis of (III) were generally low. The methoxy derivative (III) is the least promising member of the group. It was not tested for analgesic effect and, unfortunately, was not satisfactorily converted to I. Other intermediates and/or derivatives of III had no analgesic effect.



I



III

2. Synthesis of N-(2-phenylethyl) analogs of active analgesic agents. This involved BrCN N-demethylation of the active II above and its deoxy analog (1/8 as active) followed by substitution of $-CH_2CH_2Ph$ on the nitrogen. This was done in a 2-stage conversion involving Schotten-Baumann N-phenylacetylation and lithium aluminum hydride reduction of the resultant amide to amine. This appears to be superior to the normally-used, direct alkylation which gives hard-to-separate mixtures and inferior yields. Contrary to experience in the morphine, morphinan and meperidine series where activity was increased 5- to 10-fold, analgesic effectiveness was lowered in our series by replacement of methyl by phenylethyl on the nitrogen. This work will be extended to the benzmorphan series.

3. Synthesis of ethyl-2,2-diphenyl-4-morpholinobutyrate. Synthesis of this compound was undertaken at the request of the World Health Organization because the product had appeared on the market and no information was available on its addiction liability. It was tested for analgesic activity (one third that of methadone) and for addiction liability at Lexington.

10. SERIAL NO. NIAMD-64

12. BUDGET ACTIVITY: Research

13. Complementary to pharmacological investigations in this section, Serial No. NIAMD-66

14. None

16. May, E. L. "Structures Related to Morphine. V. Azabicyclodecanes derived from 2-(m-methoxyphenyl)cyclohexanone". J. Org. Chem. In press.

17. By invitation, "Analgesic activity of structures related to racemorphan", report to the Committee on Drug Addiction and Narcotics, National Research Council, 16th meeting, Sept. 30-Oct. 1, 1955.

By invitation, "Recent developments in the field of analgesia", Department of Pharmacology, George Washington University Medical School, Feb. 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases

2. Laboratory of Chemistry 3. Section on Analgesics

4. _____ 5. Serial No. NIAMD-65

6. PROJECT TITLE: Study of Analgesic Action in Humans with Pathological Pain.

7. Nathan B. Eddy 8. Joseph Cochin

9. PROJECT DESCRIPTION:

OBJECTIVES:

a) Evaluation of new analgesic agents in patients with persistent pain.

b) To determine whether or not development of tolerance and physical dependence as the result of prolonged analgesic medication can be held in abeyance by the simultaneous administration of a narcotic antagonist.

METHODS AND RESULTS:

a) By the double blind technique and the administration to the same patient of one or more rounds of a quintet of agents, which is made up of two doses of a standard analgesic, a placebo, and two doses of the new agent, we have been collaborating in the evaluation of dl-4,4-diphenyl-6-piperidine-3-heptadone (piperidyl methadone). It appears to have approximately one half of the analgesic effectiveness of morphine; that is, its effective dose is twice that of the optimal dose of morphine, but in our experience its use has been attended by the appearance of no side effects.

Twenty two patients have been made available to us by clinical sections of the National Cancer Institute.

We have been able so far, at their request, to use members of the regular nursing staff as observers for the collection of data. This has not always been satisfactory and any increase in the number of patients available for study would make the services of a full-time nurse observer essential.

b) This part of the project has not yet begun because no patients have been available. It is becoming increasingly important (see below) to get this project under way and a request has been made for the assignment of 4 beds in the Clinical Center to this purpose, patients to be admitted specifically for this study and cared for jointly by ourselves and the Metabolic Division of the Cancer Institute.

One of us (N. B. E.) has elaborated a completely detailed project outline for the study under (b), which has been very highly commended by Statistical Division. As the result of conferences here and abroad this protocol has been accepted and will be followed in joint investigation of the problem at Montefiore Hospital in New York, Eppendorfer Krankenhaus in Hamburg, Germany and at the Psychiatric Clinic in Berlin, Germany, as well as our Clinical Center. The data from all of these institutions is to be pooled and analyzed together. Hence the emphasis on the desirability of getting the project going here parallel to its pursuit at the other centers.

The clinical staff are to an increasing extent consulting us on problems of pain relief and management of patients who seem to be already tolerant to narcotic drugs.

10. SERIAL NO. NIAMD-65

12. BUDGET ACTIVITY: Research

13. Dr. Cochin collaborated with Julius Axelrod (NIMH) on "Studies on Ololuiqui, a narcotic seed of the Aztecs"; Serial No._____ and "Studies on the Biochemical Factors Involved in the Development of Tolerance to Narcotic Drugs and the Action of Narcotic Drug Antagonists", Serial No._____, and with Dr. Conan Kornetsky (NIMH) on "The Effects of Clinical and Experimental Pain on Electrical Skin Resistance", Serial No._____.

14.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
2. Laboratory of Chemistry
3. Section on Analgesics
4. _____
5. Serial No. NIAMD-66
6. PROJECT TITLE: Chemical Structure and Analgesic Action.
7. Nathan B. Eddy
8. None
9. PROJECT DESCRIPTION:

OBJECTIVES:

A long range program to ascertain the relation of chemical structure to analgesic, toxic and addictive properties and the inter-relationship of these three properties. The practical application of this work is adequate drug relief of pain with greater safety.

METHODS AND RESULTS:

By our hot plate method a large number of compounds have been screened for analgesic activity and wherever significant activity was detected a statistically evaluated ED_{50} was determined, employing the assay procedure previously described. Many of these compounds have been made in this laboratory (see reports of chemical projects), many have come from various industrial laboratories, compounds newly developed or which fitted into the general program of chemical structure and analgesic action. Also during the year we have screened a large number of compounds (more than 100) for the Chemical-Biological Coordination Center.

The outstanding development in this field is the demonstration here and elsewhere that the substitution of a phenylethyl or closely related structure for methyl on the heterocyclic nitrogen of most types of analgesic compounds enhances analgesic effectiveness with a variable effect on toxicity and addiction liability.

As temporary consultant to the World Health Organization, collaborating with a representative of the Narcotics Division of the United Nations and the Chief of the Section on Drugs Liable to Produce Addiction of WHO, two in the series of

reports for the United Nations Commission on Narcotic Drugs have been prepared. The general title is Synthetic Substances with Morphine-like Effect. The completed sections have to do with chemical structure and analgesic action and the relation of analgesic action to addiction liability. These reports will be published in the Bulletin of the World Health Organization. They are intended as authoritative reference documents for the control authorities in the field of narcotics for the Commission and the member states of United Nations.

10. SERIAL NO. NIAMD-66

12. BUDGET ACTIVITY: Research

13 through 15 = no report

16. Eddy, Nathan B. "The Phenomena of Tolerance", in the Symposium "Origins of Resistance to Toxic Agents", Academic Press, New York, 1955, pp. 223-241.

Eddy, Nathan B. "Addiction Liability: Tests and Results", read, Symposium on Analgesics, American Therapeutic Society, Atlantic City, June 3, 1955. J. Amer. Geriatric Society. In press.

Eddy, Nathan B. "The Search for new Analgesics", in Symposium on "Pain and its Relief". J. Chronic Diseases. In press.

Braenden, O. J., Eddy, Nathan B. and Halbach, H. "Synthetic Substances with Morphine-like Effect. Relationship between Chemical Structure and Analgesic Action". Bull. Wld. Hlth Org., Vol. 13, pp.937-998, 1955.

Eddy, Nathan B., Halbach, H. and Braenden, O. J., "Synthetic Substances with Morphine-like Effect. Relationship between Analgesic Action and Addiction Liability", with a discussion of the chemical structure of addiction producing substances". Bull. Wld. Hlth Org. In press.

Perrine, T. D., and Eddy, N. B. "Preparation and Analgesic Activity of 4-carbethoxy-4-phenyl-1-(2-phenylethyl)-piperidine and related compound". J. Org. Chem. In press.

17. Elected to Washington Academy of Sciences.

Member, Expert Committee on Drugs Liable to Produce Addiction, World Health Organization, Sixth Session, Geneva, Switzerland, Oct. 24-29, 1955.

Temporary consultant, World Health Organization, January 1955, November 1955.

Technical adviser, United Nations Commission on Narcotic Drugs, Tenth Session, April 1955.

Analysis of NIH Program Activities

1. NIAMD
INSTITUTE

2. Laboratory of Physical Biology
LABORATORY OR BRANCH

3. Physiology
SECTION

4.
LOCATION (IF OTHER THAN BETHESDA)

5. NIAMD-67
SERIAL NO.

6. Effects of hypoxia on physiologic mechanisms
PROJECT TITLE

7. Paul D. Altland
PRINCIPAL INVESTIGATOR(S)

8. Edwin C. Thompson, Milton Parker, Edna Devlin
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Subproject a. The fundamental mechanisms which influence altitude tolerance are unknown. Studies have been conducted to evaluate the effect of obesity on survival of rats to various simulated altitudes. Recent evidence indicates that jet pilot deaths have been due in part to fat emboli. Preliminary experiments have been conducted to study fat emboli production in rats compressed to 7.8 atmospheres followed by varying rates of decompression.

Subproject b. Experiments have been conducted to determine the effect of repeated exposure of dogs to a simulated altitude of 25,000 feet on their susceptibility to experimental bacterial endocarditis. A method for production of experimental bacterial endocarditis in dogs with aortic insufficiency has been developed.

Subproject c. The mechanism of action of the stimuli for erythropoiesis is unknown. Fundamental studies on the longevity of the erythrocyte of various species have been conducted, and the effects of cobalt and liver injections on blood formation in turtles have been determined.

Methods Employed: Repeated exposure to simulated altitudes have been conducted in 2 decompression chambers. Physiologic, cytologic, and radiobiologic methods have been used.

9. PROJECT DESCRIPTION - CONTINUED

Major Findings: Repeated exposure of obese rats to simulated altitudes of 18,000 or 25,000 feet resulted in death of more than one-half of the animals within 6 months. Rats on normal diet showed increased mortality only at 25,000 feet.

The susceptibility of dogs to experimental bacterial endocarditis was found to be moderately increased by repeated exposure to simulated altitudes of 25,000 feet. The susceptibility of dogs to acute and subacute experimental endocarditis was found to be greatly increased by production of aortic insufficiency by perforating aortic leaflets with a punch introduced through an incision in the ascending aorta.

By use of glycine-2-C^{14} it has been found that the longevity of the turtle (reptile) erythrocyte exceeds 500 days, and that the toad (amphibian) exceeds 270 days. These findings support the hypothesis that erythrocyte longevity of poikilotherms may be correlated with species longevity. Cobalt used in varying dosages failed to stimulate erythropoiesis in turtles; this contradicts the current concept prevalent in medical literature that cobalt acts as an erythropoietic stimulus in all vertebrate animals.

Significance to NIAMD Research: Contributes facts concerning the importance of fat in survival of animals exposed to reduced oxygen tensions. Provides data on the reaction of dogs to severe hypoxic stress and supplies a method for production and study of cardiovascular diseases in dogs. Gives data on factors involved in blood formation and erythrocyte longevity in an area of comparative physiology previously unstudied.

Proposed course of project: Studies on mechanism involved in altitude tolerance. Experiments will be conducted on the effect of exposure of obese rats to a simulated altitude of 18,000 feet with emphasis on survival and pathology; study of the influence of age, convulsions, time of decompression and strain differences upon the production of fat embolism; study of influence of hypoxia on the physiology of reproduction in the male dog. In addition, physiologic studies on the hypoxic tolerance of animals with cardiovascular defects (aortic insufficiency) will be initiated, and experiments on bacterial endocarditis in dogs will be continued. Experiments on erythrocyte longevity in poikilotherms will be continued.

NIAMD-67
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Studies with obese animals and the relation of compression-decompression to production of fat emboli are being conducted with cooperation of Dr. Olaf Mickelsen of Section of Biochemistry and Physiology of Nutrition, NIAMD, and Dr. Webb Haymaker, of Department of Pathology, Armed Forces Institute of Pathology.

Studies with dogs with aortic insufficiency and endocarditis are being conducted with Dr. Joseph Roshe, Surgery Branch, National Heart Institute and Dr. Benjamin Highman, Section on Histochemistry and Pathology, NIAMD.

Studies on longevity of erythrocytes are being conducted with cooperation of Dr. Kirkland Brace of General Radiobiology Section, National Cancer Institute.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OF FUNDS), IDENTIFY SUCH RESEARCH:

No parallel research in PHS.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Altland, Paul D. and M. Parker. Effects of hypoxia upon the box turtle. Am. J. Physiol., 180: (2) 421-427, 1955.

Highman, B. and P. D. Altland. Effect of altitude and cobalt polycythemia, hypoxia and cortisone on susceptibility of rats to endocarditis. Circulation Research, 3: (4) 351-356, 1955.

Brace, K. and P. D. Altland. Red cell survival in the turtle. Am. J. Physiol., 183: (1) 91-94, 1955.

Highman, B., J. Roshe and P. D. Altland. Production of endocarditis with Staphylococcus aureus and Streptococcus mitis in dogs with aortic insufficiency. Accepted for publication by Circulation Research.

NIAMD-67
SERIAL NO.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

Analysis of NIH Program Activities

1. NIAMD (INSTITUTE) 2. Laboratory of Physical Biology

3. Physiology (SECTION) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NIAMD-68 (SERIAL NO.)

6. The effect of roentgen radiation on tissue growth and differentiation
PROJECT TITLE

7. Helen D. Park
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To study patterns of growth and differentiation in simple tissues after various doses of roentgen radiation.

Methods Employed: Hydra, a fresh water coelenterate, has been used in these studies because of its ability to regenerate (differentiate) lost parts and because during asexual reproduction growth phases can be relatively easily separated from differentiative phases of development.

Major Findings: (1) That hydras exposed to 15,000 r + 15,000 r of X-rays 1-24 hours later are much less sensitive to the first exposure, using viability as the criterion of effect. The decrease in sensitivity varies directly with the interval between exposures. The effect produced by 15,000 r + 15,000 r 24 hours later is approximately one-tenth that produced by a single exposure to 30,000 r, (2) that short exposures to low doses of X-rays did not stimulate growth (by budding) of hydras.

Significance to NIAMD Research: A relatively simple, asexually reproducing organism can be desensitized to a growth - inhibiting dose of X-rays by a previous exposure to a low dose of the radiation.

Proposed course of project: (1) To study the effects of long exposures to low doses of ionizing radiations on respiration and growth of hydras. (2) To assist in other work of the unit on insect respiration in relation to diabetes.

NIAMD-68
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956.

No cooperating units.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH.

No parallel research in PHS.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

Analysis of NIH Program Activities

1. NIAMD (INSTITUTE) 2. Laboratory of Physical Biology (LABORATORY OR BRANCH)

3. Physiology (SECTION) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NIAMD-69 (SERIAL NO.)

6. The circulatory response to injection of the synthetic plasma expanders, dextran and polyvinylpyrrolidone. (PROJECT TITLE)

7. Louise H. Marshall (PRINCIPAL INVESTIGATOR(S))

8. Charles H. Hanna (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives: Preliminary to investigation of the effect of dextran and polyvinylpyrrolidone (PVP) on renal function in dogs, it has been necessary to study the circulatory changes that occur after their injection in both sensitive and non-sensitive species. Arterial blood pressure was measured continuously when the expanders were administered to dogs, rabbits, hamsters, rats and guinea pigs. In addition, capillary circulation has been studied to elucidate the changes occurring in permeability and flow that might account for the arterial pressure responses in sensitive species.

Methods Employed: Arterial blood pressures were measured with a sensitive strain gage. Capillary permeability was studied by means of the isolated hind leg perfusion technique. In these experiments flow and weight variations were measured while the amputated limb was perfused at constant pressure with various artificial mixtures. In addition, capillary flow in the mesocecum and hind foot web of the rat were followed by direct microscopic visualization under conditions of constant temperature and moisture.

Major Findings: A detailed study of the carotid blood pressure in the guinea pig was made since none appears in the literature. In this species it was found that a smaller portion of the cardiac cycle is regularly taken up by filling and coronary circulation than is the case in other small mammals.

9. PROJECT DESCRIPTION - CONTINUED

Major Findings (Continued):

In dogs injected with PVP and in rats with dextran it was found that the arterial blood pressure immediately falls, recovers partially, and then more slowly decreases to low levels, when signs of edema and flushing may occur. In non-sensitive species there is a rise in blood pressure or no change, which is also the case if a previously sensitive animal is given a second injection.

Isolated dog or rat limb perfusions showed striking changes in flow and weight gain when the perfusion mixture contained PVP or dextran respectively. Other species were relatively unresponsive. Abrupt stoppage of the circulation in the rat's web was noted after dextran injection, but not in the mesocecum.

Significance to NIAMD Research: These studies will facilitate interpretation of the effects of the plasma expanders on renal function. Correlation with changes at the cellular level contributes to an understanding of renal and circulatory hemodynamics.

Proposed course of project: The effect of PVP on circulation in the dog's mesentery will be studied. Plans are being made to undertake the study of renal function during the stress of excretion of large amounts of the synthetic colloids used as plasma expanders.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956.

No cooperating units.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No parallel research in PHS.

NIAMD-69
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Hanna, C. H. and L. H. Marshall. Effect of sampling on erythrocyte, dye and protein concentration of venous blood of dogs. Am. J. Physiol., 182: 331-336, 1955.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

Analysis of NIH Program Activities

1. NIAMD (INSTITUTE) 2. Laboratory of Physical Biology (LABORATORY OR BRANCH)

3. Physiology (SECTION) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NIAMD-70 (SERIAL NO.)

6. Physics of gas diffusion into and through tissues
PROJECT TITLE

7. John B. Buck
PRINCIPAL INVESTIGATOR(S)

8. Margaret L. Keister
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Our objective is to learn as much as possible about the physical factors which govern the passage of respiratory gases through biological structures, particularly in the gas phase and in the region of phase change, where gases enter and leave tissues. This involves the study of tissue permeability, mechanism of diffusion, and respiratory responses to various gas concentration gradients.

Methods Employed: We have used insect material because it is amenable to much more drastic experimental alteration than that of any other air-breathing animal, and yet resembles lung-breathing vertebrates in having a direct tissue-gas exchange. Furthermore, in many instances respiration has been simplified to pure diffusive transfer, which is thus susceptible to precise analysis.

Major Findings: (1) Fly larvae were found to die more quickly under oxygenated water than aerated water, even though their oxygen uptakes were much higher. The effect was explained on relative gas interdiffusion rates plus flooding of respiratory passages by tissue fluids. (In press.) (2) The first appearance of gas in the respiratory passages, which normally occurs just after molting but may be induced prematurely by exposure to a critical low ambient oxygen concentration, was explained by a combination of metabolic uptake of liquid through the respiratory epithelium and relative interdiffusion rates of the gases involved. (In press.)

9. PROJECT DESCRIPTION - CONTINUED

Significance to NIAMD Research: From the standpoint of basic knowledge our studies have contributed to the understanding of gas-liquid phase exchange in respiration. From the standpoint of practical human physiology, our work may have some bearing on passage of liquid across respiratory epithelia as occurs (a) in the normal clearing of the lung of fluid just after birth, and (b) pathological transudation, as in pulmonary edema.

Proposed course of project: Future projects planned include (a) investigation of failure of diapausing pupae to accumulate an oxygen debt, (b) diffusive O_2 transport and tissue pO_2 in hypoxia. In addition, with an enlarged group it is proposed to investigate the intermediary metabolism of carbohydrate in insects in relation to diabetes. The line of reasoning is as follows: (a) insect muscle has an intrinsic metabolic rate 10 times that of the most active mammalian muscle, (b) insect flight muscle can be instantaneously stimulated 100-fold or more in metabolic rate, (c) insect muscle in general uses the same metabolic fuels and pathways as mammalian muscle, (d) insulin has been reported to lower insect blood sugar. It therefore appears possible that, in the first place, insects might be used in a bioassay method for insulin (which is badly needed), and in the second place, that a study of the metabolic utilization of carbohydrate might yield clues to the diabetic defect. It is proposed to carry on parallel biochemical and respirometric studies of glycogen flux and utilization of various sugars under conditions of rest, flight and insulin administration.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956.

No cooperating units.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH.

No parallel research in PHS.

NIAMD-70
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Buck, J. B. and M. L. Keister. Cyclic CO_2 release in diapausing Agapema pupae. Biol. Bull., 109: (1) 144-163, Aug., 1955.

Buck, J. B. Some reflections on the control of bioluminescence. The Luminescence of Biological Systems, pp. 323-332, publication of AAAS, 1955.

Buck, J. and M. L. Keister. Observations on the physiology of parasitized saturniid pupae. IN PRESS (Annals of Entomol. Soc. Am.).

Buck, J. B. and M. L. Keister. Further studies on gas-filling in the insect tracheal system. IN PRESS (J. Exptl. Biol.).

Buck, J. B. and M. L. Keister. Diffusion phenomena in the tracheae of Phormia larvae. IN PRESS (Physiol. Zool.).

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1954:

To J. B. Buck:

Elected to Council, Society of General Physiologists, 1955-57.
Elected Vice-President, Am. Soc. of Zoologists, 1955.

Analysis of NIH Program Activities

1. NIAMD (INSTITUTE) 2. Laboratory of Physical Biology (LABORATORY OF BRANCH)

3. Physiology (SECTION) 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NIAMD-71 (SERIAL NO.)

6. Pulmonary ventilation
PROJECT TITLE

7. Heinz Specht
PRINCIPAL INVESTIGATOR(S)

8. Howard F. Brubach
Loyal G. Goff
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

The studies in pulmonary ventilation at this Laboratory have resulted from observations made in low and normal density atmospheres. Studies in high density atmospheres are now in progress as embodied in a subproject on the physiology of underwater swimmers.

Objectives: The principal objective of the over-all project is to explore new phenomena regarding breathing behavior with regard to their physiological significance. In order to accomplish this various studies of physiology must be undertaken in order to accurately control the experimental situation. Thus, in underwater swimmers it is hoped that ultimately breath velocity patterns at various depths may yield information that can be compared with our studies at high altitude. As interim objectives the physiology of finned underwater swimmers is being studied on contract to the Office of Naval Research. This is the third year of such support.

Methods Employed: Subjects are studied for oxygen consumption, CO_2 production, breath velocity patterns, and other responses as necessary while swimming or resting immersed under accurately known conditions of temperature, water speed, drag, etc. Since conditions under water are less amenable to volumetric gas measurement than in the classical metabolic studies in air it has been necessary to devise special equipment for the various measurements. In addition, swimming facilities had to be set up for use in the

9. PROJECT DESCRIPTION - CONTINUED

Methods Employed (Continued):

laboratory as an alternate to the use of the circulating water channel of the David Taylor Model Basin which was placed at our disposal for limited periods in the past.

Major Findings: The average efficiency of underwater swimming in this series did not exceed 5.1%. This was calculated on the basis of the differences between oxygen consumption during the drag measurements when swimmer activity was at a minimum and the oxygen consumption at known swim rates, compared with the actual work accomplished as determined by the drag data. At the highest swim rate (1.0 knot) the average work accomplished was about 800 ft.-lb./min. This corresponds to light work in air. The measurements of oxygen consumption, respiratory minute volume and breath velocity, however, fall well within the known ranges for heavy work in air. A marked influence of breathing pattern and ventilation rates on canister efficiency in the removal of CO_2 was noted. Personnel who have had extensive training in diving and underwater swimming appear to maintain a high alveolar CO_2 tension during underwater exercise.

For long distance swimming a rate of 0.7 knots seems to be optimum under the conditions of this series of tests. At this rate swimming efficiency was maximum (5.1%) as was the coefficient of oxygen utilization (48 cc/liter of ventilation) and the calculated oxygen consumption per mile was lowest (approximately 70 l.).

Significance to NIAMD Research: In order to extend the understanding of both normal and abnormal respiratory gas exchange it has become necessary to explore the significance of phenomena beyond those used classically for measuring pulmonary function. Inasmuch as physical environment affects such phenomena the study of atmospheric density effects on breathing will ultimately form a new set of criteria for judging the level of normal or abnormal behavior.

Proposed course of project: The contract with ONR is entering the last year. Because of the Navy's continuing interest in the practical aspects of these problems, extension of the contract beyond the present period may be found desirable. It is planned to obtain more comprehensive data on breath velocities under different physical environmental conditions. The studies of artificially mixed atmospheres will be continued using Freon 114 in lieu of sulfur hexaflouride. Toxicological difficulties encountered with the latter make it undesirable for this purpose.

NIAMD-71
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

This project is being carried partly by funds from the Office of Naval Research during 1955 and 1956.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No parallel research in PHS.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Specht, H. Hydrodynamics and Propulsion of the Unaided Swimmer. Report presented to the meeting of the Panel on Underwater Swimmers and will appear in the National Academy of Sciences - National Research Council publication NRC:CUW:0221 (SECRET).

Brubach, H. F. Desiccator cover remover and sleeve wrench. Sci., 122: (3173) 761-762, Oct. 21, 1955.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Brubach, Howard F. Incentive award ($200.00) for the development of two instruments for the safe, efficient removal of desiccator lids.

Analysis of NIH Program Activities

1. NIAMD — INSTITUTE
2. Laboratory of Physical Biology — LABORATORY OR BRANCH
3. Photobiology — SECTION
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NIAMD-72 — SERIAL NO.

6. Effects of radiant energy on the oxidative metabolism and chemosynthesis in living cells — PROJECT TITLE

7. Dr. R. A. Olson — PRINCIPAL INVESTIGATOR(S)

8. R. Crickard, E. Engel — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To study the specific effects of radiant energy on cellular oxidative metabolism and chemosynthesis utilizing the ultraviolet, visible, and infrared regions of the spectrum. To determine the role and identity of enzymes, photosensitizers, and pigments in the photochemical and other reactions involved. In addition, to identify the intracellular morphological changes associated with such metabolic effects in order to localize reaction sites and participating cell structures or inclusions.

Methods Employed: Cell suspensions of unicellular organisms (Chlorella, etc.) cultured aseptically under various experimental conditions are irradiated in monochromator systems designed to provide homogeneous radiation of all cells. Simultaneous measurements of oxygen concentration and the rate of oxygen metabolism during prescribed radiation and dark intervals under various conditions of intensity, wavelength, dark adaptation substrate availability, pigment density and specific enzyme inhibitors are provided at 3-second intervals by a fast electronic pen recording polorographic instrument developed for this purpose. (See Brackett.) These measurements when correlated with optical determinations in these cells of the concomitant transient changes in energy absorbed, selective absorption, quenching of fluorescence, and luminescence due to back reaction provide quantum and kinetic data leading to the identity and nature of the

9. PROJECT DESCRIPTION - CONTINUED

Methods Employed (Continued):

photochemical and dark reactions involved. High-resolution monochromatic photomicrography combined with histochemical techniques provide morphological data concerning the sites of action within the cell.

Major Findings: Particular attention has been centered upon the characteristic rate changes in oxygen metabolism associated with the nonsteady state occurring in cell suspensions following the dark-light and light-dark transitions. Quantitative recording of these rate changes with a time resolution (3 seconds) unapproached by other methods has revealed a number of related, unreported effects which point toward the clarification of the sequential role of various metabolic intermediates and related changes in their respective reservoirs. Some of these effects and their significance are described in the following.

There occurs on the illumination of aerobic suspensions an immediate burst of oxygen, reaching a maximum up to that of the normal rate in 6-9 seconds, which appears superimposed on the time-course curve of the normal sustained rate. Anaerobically dark adapted suspensions show, during the resulting delay of continuous oxygen evolution, a similar burst which is followed, after brief oxygen uptake, by a smaller but more prolonged secondary burst. The O_2 burst occurring after anaerobic dark adaptation is dependent upon the immediately prior rate of dark oxidative metabolism permitted by increasing the oxygen concentration just before illumination (limited below 0.1% oxygen) while the burst occurring after aerobic dark adaptation is dependent upon the rate of respiration permitted by the endogenous level of some photochemically produced substrate. This suggests that the O_2 burst reaction is controlled by the reservoir of some intermediate product of dark oxidative metabolism which after long absence of carboxylation or the absence of oxygen is depleted by side reactions associated with non-oxidative endogenous energy transfer. Since the O_2 burst effects are unaffected by low CO_2 concentrations (less than 0.5%) which markedly depress the subsequent sustained rate, they appear to be independent of carboxylation. Enhancement of the CO_2 limited sustained rate of O_2 evolution by benzaldehyde (10^{-5} molar) without noticeable effect on the O_2 burst suggests a reaction with some native hydrogen acceptor which becomes depleted in the absence of carboxylation. An immediate O_2 uptake or negative burst (oxidative

NIAMD-72
SERIAL NO.

9. PROJECT DESCRIPTION - CONTINUED

Major Findings (Continued):

metabolism) occurring aerobically just after the light-dark transition also appears to be independent of immediately previous carboxylation, in spite of such a dependency of the aerobic burst. This uptake corresponds to the period following the light-dark transition of no detectable CO_2 exchange and luminescence caused by back-reaction reported in the literature. The interdependence of the early dark oxidation and the secondary burst effect seen under certain conditions which lead to a merging of the two bursts suggests a delayed energy-competing non-oxygen process as the cause separating the two bursts. Merging of the two bursts would occur on saturation of this pool. Evaluation of the full significance of these effects is not feasible since further data indicating the dependence of these effects upon numerous other variables and upon other transients are not yet available.

Significance to NIAMD Research: Unicellular organisms with special metabolic adaptation for the utilization of radiant energy in chemosynthesis carry out to a marked degree but by different pathways the same general metabolic reactions which occur in the more specialized cells of higher organisms. During the synthesis of proteins, fats, oils, steroids and other cell components some mechanism for CO_2 assimilation is now considered to be operable in the normal metabolism of all cells. In cells not adapted to utilize radiant energy the energy source for this process is provided chemically via phosphate or other energy transferring systems. Radiant energy thereby becomes a tool in photosynthetic organisms by which specific metabolic pathways may be induced at will without changing in any other way the physical or chemical environment of the cell. Use of the appropriate wavelength and intensity of radiation may provide considerable choice in the degree of participation by known pigments in both synthesis and oxidative reactions while the duration and intensity of light allows for a kinetic study of the carboxylation-decarboxylation equilibrium. Information in this direction leads to a better understanding of the more subtle metabolic pathways which characterize anomalies in physiologically diseased cells.

NIAMD-72
SERIAL NO.

9. PROJECT DESCRIPTION - CONTINUED

Proposed Course of Project: Immediate emphasis on further study of the effect on O_2 transients of physical factors (intensity, wavelength, temperature, etc.) and physiological factors (O_2 and CO_2 concentration, glucose and other substrate concentrations, and specific inhibitors). Determination of possible linkage of transients with dark oxidative phosphorylation and photo-phosphorylation via specific effects of phosphorylating decoupling agents. Determination of all these factors on the behavior of other transients (CO_2, quenching of fluorescence, luminescence, etc.) and subsequent correlation with O_2 transients. Substitution of cell particulate components (chloroplasts, etc.) for intact cells in above studies in order to isolate specific component reactions and to allow addition of various phosphoric esters unable to penetrate the intact cell. Continuation of cellular morphological studies and histochemical techniques for the determination of cellular sites of action.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

No cooperating units.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No parallel research in PHS.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Olson, R. A. Physiological action of auxin. Physiol. Rev., 35: (1) 57-89, Jan., 1955.

NIAMD-72
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955 (Continued):

Brackett, F. S., R. A. Olson and R. G. Crickard. Transients in O_2 evolution by Chlorella during light and dark. I.

Olson, R. A., F. S. Brackett and R. G. Crickard. Transients in O_2 evolution by Chlorella during light and dark. II.

(Above papers included by invitation in "Gatlinburg Photosynthesis Conference 1955", edited by H. Gaffron, to be published in book form after revision.)

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

Analysis of NIH Program Activities

1. NIAMD — INSTITUTE
2. Laboratory of Physical Biology — LABORATORY OR BRANCH
3. Photobiology — SECTION
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NIAMD-73 — SERIAL NO.

6. Investigations of the action of radiant energy on biologically important compounds, with emphasis on steroids. — PROJECT TITLE

7. Dr. Norman E. Sharpless — PRINCIPAL INVESTIGATOR(S)

8. Mrs. Janet S. Munday — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objectives of this project are the establishment of the structure of certain intermediates in the photochemistry of steroids and to study the kinetics and quantum efficiences in the formation of alteration products.

Methods Employed: Ultraviolet radiation is the tool employed to effect the alterations in the materials and spectroscopy in the ultraviolet and infrared to evaluate the changes resulting.

Major Findings: The irradiation of ergosterol with λ = 2537A light is found to yield as much as 70% tachysterol and 16% calciferol. In a particular arrangement developed for irradiating substantial quantities of steroids the yield of tachysterol as a function of ergosterol concentrations rises rapidly to a maximum at an ergosterol concentration of 10 mg/liter, after which it falls at a much slower rate. Also, under these conditions the yields are independent of the volume of the solution.

The new tools and techniques available for infrared spectrometry, particularly the potassium bromide pellet technique, have greatly increased the versatility and usefulness of infrared methods. The pellet technique is particularly important for solid organic compounds, since it eliminates

the interference of the bands of the mulling agent as well as eliminating the dissolving of part of the sample by the mulling medium. These techniques are particularly valuable in steroid research, permitting investigation of hydrogen bonding in these molecules. A preliminary investigation of such solid and solution spectra has been undertaken, using vanillin as a model compound. This is a uniquely useful compound for this type of research, since it is stable, low melting, soluble in water as well as most organic solvents, and contains a number of important structural features.

The data obtained thus far on vanillin may be divided into three groups: solid spectra (pellet, film) "inert" solvents (CCl_4, CS_2, C_6H_6, etc.) and "reactive" solvents (pyridine, ether, etc.). Of principal interest are those bands due to free OH groups, bonded OH and the carbonyl group. In the inert solvent group the free OH bands are sharp and lie between 3520 and 3592 cm^{-1}, while the bands due to bonded OH are much less intense, broader and lie between 3385 and 3496 cm^{-1}. The "reactive" solvent spectra show no evidence of free OH bands, while the bonded OH bands lie between 3328 and 3468 cm^{-1}. The spectra of the solids likewise show no bands due to free OH, while maxima of the broad bands due to bonded OH are in the vicinity of 3235 cm^{-1}.

The carbonyl bands are reasonably constant for both the inert and reactive solvents, lying between 1691 and 1707 cm^{-1} with one (cyclohexane) at 1718 cm^{-1}. However, the carbonyl bands for the solid states are displaced to lower frequencies at 1676 cm^{-1}.

A valuable group of reagents in biological chemistry are certain salts of 12-heteropoly acids (phosphomolybdic acid for blood sugars and steroids, silicotungstic acid for steroids). In addition, these compounds are also useful as analytical forms for the central atoms, such as phosphorous and silica in biological fluids. It was therefore felt of interest to catalog infrared spectra of some representative compounds of this series. Although extremely complex compounds of high molecular weight, these molecules show remarkably simple spectra in the sodium chloride region, usually four or five bands in the 10-16 micron region. A report is being prepared for publication on these compounds.

Significance to NIAMD Research: Fundamental investigations into the structures and reactivities of biologically important molecules are basic to the understanding of their actions in the cell or on the organism, as well as being necessary for the development of similarly acting substances or antagonists.

Proposed Course of Project: A comprehensive continuation of the kinetics and mechanism of the vitamin D series, transformation, utilizing infrared and ultraviolet spectroscopy for establishing the structures of various intermediates and related compounds.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

No cooperating units.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No parallel research.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Sharpless, N. E., A. E. Blair and C. R. Maxwell. "The Effect of Ionizing Radiation on Amino Acids. II. The Effect of X-Rays on Aqueous Solutions of Alanine." Radiation Research 2, 135 (1955).

Sharpless, N. E., A. E. Blair and C. R. Maxwell. "The Effect of Ionizing Radiation on Amino Acids. IV. pH Effects on the Radiation Decomposition of Alanine." Radiation Research. In press.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

No honors or awards.

Analysis of NIH Program Activities

1. NIAMD — INSTITUTE
2. Laboratory of Physical Biology — LABORATORY OR BRANCH
3. Photobiology — SECTION
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NIAMD-7L — SERIAL NO.

6. Ionization studies in the upper atmosphere.
PROJECT TITLE

7. Dr. Herman Yagoda
PRINCIPAL INVESTIGATOR (S)

8. H. B. Smith, Jr. (until June 1955); H. Crane (from Oct. 3, 1955).
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Evaluation of the flux, energy and charge spectra of the heavy primary nuclei of the cosmic radiation.

Methods Employed: Exposure of nuclear emulsions in rockets and high altitude balloons (skyhooks).

Major Findings: Successful recovery of emulsions flown to 137 miles in Viking Rocket No. 9 has permitted an evaluation of the flux and charge spectrum of the heavy primary nuclei entering the atmosphere. A report covering techniques and observations has been accepted for publication in the Canadian J. of Research, Jan. 1956.

A new cosmic ray phenomenon in which a neutral particle decays with the formation of a pair of positive and negative π-mesons has been reported in the Physical Review 98, 103 (1955).

Significance to NIAMD Research: Basic investigations in nuclear physics which may have significance of military or health importance.

Proposed Course of Project: Emulsions have been flown and recovered mounted in a phantom of a human head (see Dec. 1954 report). This material is now under microscopic observation, and when completed will furnish invaluable data for evaluating the hazard from thindown hits by heavy primaries in tissue.

10. NIAMD-74
SERIAL NO.

12.
BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Naval Research Laboratory Rocket Sonde.
Office of Naval Research Balloon Project.
U. S. Air Force Space Medicine Laboratory.
Rand Corporation, Santa Monica, California.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No parallel research.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

H. Yagoda. Isothermal processing of thick nuclear emulsions. Rev. Scientific Inst. 26, 263-266 (1955).

H. Yagoda. Pion-pair production in a nuclear emulsion. Physical Rev. 98, 103-104 (1955).

H. Yagoda. Mesonic-decay of an ejected triton. Physical Rev. 98, 153-157 (1955).

H. Yagoda. Electrographic and Contact Printing Methods for the study of cosmic dust impacts on mesospheric vehicles. Rand Corporation Symposium on High-Speed Impact Phenomena, Santa Monica, California. Classification - Secret.

H. Yagoda. Book Review of "Autoradiography in Biology and Medicine," by G. A. Boyd. Science 122, 520-521 (1955).

H. Yagoda. Observations on stars and heavy primaries recorded in emulsions flown in Viking Rocket No. 9. Canadian J. of Research, in press, 47 pp. plus 16 illust.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Consultant for the Rand Corporation on meteoritic impact phenomena.

Analysis of NIH Program Activities

1. NIAMD — INSTITUTE
2. Laboratory of Physical Biology — LABORATORY OR BRANCH
3. Photobiology — SECTION
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NIAMD-75 — SERIAL NO.

6. Mechanism of Photo Action.
PROJECT TITLE

7. Dr. F. S. Brackett
PRINCIPAL INVESTIGATOR(S)

Molecular structure - Dr. Urner Liddel
Cellular action - Dr. Rodney Olson
Molecular action - Dr. Norman Sharpless
8. OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To gain an understanding of the mechanism by which radiation affects living cells.

Emphasis is placed upon processes in which radiation contributes essentially to the requirements of living cells (in contrast to the destructive actions of photo-oxidation and photo-ionization).

Important mechanisms of particular concern are:

Hydrogen transfer and acceptance.
Carboxylation.
Phosphorylation.
Photo-isomerization.

The approach to these broad objectives is through a series of projects of more specific and limited objectives in association with the investigators indicated.

While considerable progress has been attained in the study of the action of radiation on living cells--particularly photosynthesis (with Dr. Olson)--other

9. PROJECT DESCRIPTION - CONTINUED

aspects of the project are in the preparatory stages which involve purchase of equipment, development of instruments, and perfection of methods.

Methods Employed: Much of our research requires special instruments designed and developed in this laboratory. Thus the study of transients in oxygen exchange (with Dr. Olson) is possible because of the development of an instrument which records not only the oxygen concentration in a half milliliter of cell suspension to .1 mμ mole/ml but also computes and directly records the rate of change of oxygen concentration to 1 mμ mole/ml/min with a time resolution of 3 seconds. A grating monocromator is being constructed to replace the prismatic system used previously.

Similarly, in studies of molecular structure (Liddel) and molecular action (Sharpless) a major portion of the instruments are of our own design and construction.

The development of infrared facilities has extended over several years. a) An instrument for study of hydrogen bonding at high resolution in the region of 3 μ was finally delivered by the Central Instrument Shop. This was started before the war and resumed soon after. A large part of this year has been devoted to rebuilding elements of incomplete or faulty construction. However, we are now obtaining preliminary recordings. b) An instrument for the visible and near infrared to about 2.4 μ has reached the state of partial usefulness. c) The instrument developed by conversion of an early Perkin-Elmer Model 12 has been used in a number of preliminary studies (see Sharpless and Liddel). d) A Perkin-Elmer Model 13 with microscope attachment has been received, secondary facilities developed, and is now in fully productive use (see Liddel). Plans have been developed and construction is under way to convert this instrument: 1) to linear frequency recording instead of prismatic; 2) optical density recording instead of transmission; and 3)varying rate of recording according to error requirements.

(These are improvements developed in this laboratory and are now incorporated in only a few commercial instruments.)

9. PROJECT DESCRIPTION - CONTINUED

With these instrumental developments nearing completion the emphasis is being shifted from instrumentation to a consideration of the basic questions of molecular and photochemical significance. Thus, Dr. Liddel, whose interest is molecular structure, replaces Dr. Daniel, whose interest was electronics. A well rounded study of radiation mechanism should include more emphasis on the ultraviolet and on quantum theoretical chemical physics.

Major Findings:

See associated projects

Significance to NIAMD Research: A basic understanding of the mechanism of the action of radiation in cells would answer some of the most fundamental problems of living things, such as how energy is stored and made available. Almost as fundamental is the role of radiation in steroid transformation, particularly production of Vitamin D, which appears to be a very general requirement. Hydrogen bonds are no doubt of key importance in biological processes. While our findings appear remote from immediate medical practice, advances in biological understanding usually bear fruit sooner than one can anticipate.

Proposed Course of the Project: Future plans are considered under the more specific projects. It is hoped that progress in these particular lines will in the future contribute to the more basic objectives of this parent project.

10. NIAMD-75
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956.

No cooperating units.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No parallel research in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

1. NIAMD — INSTITUTE
2. Laboratory of Physical Biology — LABORATORY OR BRANCH
3. Photobiology — SECTION
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NIAMD-76 — SERIAL NO.

6. Molecular structure determined by spectroscopic methods. — PROJECT TITLE

7. Urner Liddel — PRINCIPAL INVESTIGATOR(S)

8. Edwin D. Becker — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: (1) An understanding of the forces between atoms in molecules of biological importance. (2) Development of precision analytical methods for certain components of molecules of biological importance.

Methods Employed: Absorption of light in the infrared region of the spectrum has long been interpreted as arising from motions of atoms in molecules. Certain bands have been associated with certain molecular groupings; e.g. OH, NH_2, CH_3 radicals, etc. Thus an analysis of a chemical compound may be made. Under certain conditions, this analysis can be quantitative.

As the environment of a molecule is changed in dielectric character, the absorption is changed. Molecules such as alcohols "associate," i.e. form agglomerates or polymers, so that the absorption is varied. These associations take place through "hydrogen bonds." Thus a quantitative study of the absorption leads to a knowledge of the characteristics of the hydrogen bonds.

Major Findings: The "hydrogen bonding" between methanol molecules and between ethanol molecules is strongly influenced by concentration, even in the range less than 1 molar, in carbon tetrachloride solution. Of more interest is the variation in the bonding as a function of temperature. Addition of certain other substances (e.g. acetone) to the solution has a profound effect on hydrogen bonding.

Significance to NIAMD Research: If the protein molecule is held together with hydrogen bonds, and if these bonds are sensitive to temperature and chemical environment (as we know proteins to be) this work may lead to an understanding of the forces involved in proteins, explain their temperature dependence, and give a lead as to the types of chemical groupings that might be added to control changes in protein molecules.

It is believed that a knowledge of the characteristic changes of hydrogen bonding will lead to a much better understanding of biological processes.

Proposed Course of Project: Quantitative analysis of the spectra of molecules exhibiting hydrogen bonding, together with the effects of addition of other substances on this absorption.

Extension of the program from OH-containing molecules to NH-containing molecules, and complex structures.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

No cooperating units.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No parallel research.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

No publications.

NIAMD-76
SERIAL NO.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Election to Washington Academy of Sciences.
Election to Board of Directors, American Nuclear Society.
Election to Executive Committee, Board of Directors, American Nuclear Society.
Special award by Board of Directors, American Nuclear Society for initial conception of need for the Society and action which led to formal organization of the Society.
Award of the grade of "Fellow" by the Board of Directors of the Institute of Radio Engineers for the initiation, development, and support of a national program in nuclear physics research.

Analysis of NIH Program Activities

Project Description Sheet

1. NIAMD — INSTITUTE
2. Laboratory of Physical Biology — LABORATORY OR BRANCH
3. Molecular Biophysics — SECTION
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NIAMD-77 — SERIAL NO.

6. The physical chemistry of membranes and complex membrane systems of biological interest. — PROJECT TITLE

7. Dr. Karl Sollner — PRINCIPAL INVESTIGATOR(S)

8. Dr. Rex Neihof (went on leave of absence as of Sept. 13, 1955); Dr. Sheldon Dray (transferred as of Sept. 19, 1955, to Nat. Micro. Inst.); Dr. Joseph Wagner (guest worker until September 7, 1955; thereafter, Visiting Scientist); Dr. Norman Gershfeld (started work on November 21, 1955); Dr. Marc Lewis, Post-Doctorate Fellow (started work on Aug. 15, 1955. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: A physicochemical study of membranes and membrane model systems with the purpose of providing a rational physicochemical basis for the elucidation of numerous phenomena in living organisms, for instance, electrolyte balance and electrolyte distribution, the accumulation of electrolytes in living cells, cell and nerve potentials, and electrophysiology in general.

Methods Employed: The preparation of membranes of highly characteristic and specific electrochemical properties (the methods having been worked out by the principal investigator and his collaborators), and the investigation of these membranes, and of membrane systems in which such membranes are the functional part, by physicochemical, especially electrochemical methods, such as potential and resistance measurements, also by chemical analytical procedures, including radioactive tracer methods.

Major Findings: A theory was worked out in detail which explains on a strictly physicochemical basis the differential rates of exchange of two or more coexisting species of ions of the same charge across permselective and similar membranes. The ratio of the rates, that is the ratio of the fluxes across the

membrane, of any two species of simultaneously exchanging ions, can be calculated from their relative concentrations and an independent electrometric measurement, namely the measurement of the bi-ionic potential which arises with the same two species of ions across the same membrane. The predictions of the theory have been verified by quantitative studies in a variety of systems. These observations could furnish the basis for an understanding of the preferential accumulation by living cells, by means of an aqueous mechanism, of such ions as potassium over sodium or iodide over chloride. (Neihof and Sollner).

It was postulated on a theoretical basis and proven experimentally that the concentration of one (or more) species of ions of the same charge in a membrane system which drifts towards a Gibbs-Donnan membrane equilibrium may temporarily reach a magnitude far in excess of that established in the state of membrane equilibrium. This effect is particularly large if the ions under consideration give large flux rates under the conditions of the above indicated experiments and if the intrinsically more permeable species of ions is present in a low relative concentration. Thus, in dynamic membrane systems (most living cells and tissues are of this type) concentrations may be reached which cannot be explained by the conventional application of the theory of membrane equilibria. (Neihof and Sollner).

Strong-base type electropositive collodion matrix membranes of extreme electrochemical activity were prepared by the adsorption of the polyelectrolyte poly-2-vinyl-N-methyl pyridinium bromide from aqueous solutions on preformed highly porous collodion membranes whose porosity can subsequently be reduced by drying. These membranes combine the mechanical strength, flexibility, and thinness characteristic of collodion membranes with a very low resistance and an extreme degree of ionic selectivity--the anions being the preferred ions--which in 0.01 N solution may reach values of 20,000 or 30,000 to one. These membranes are a tool of great promise in the study of complex membrane systems. (Gottlieb, Neihof, and Sollner.)

Theoretical work was continued on a recently (1955) published theory of the accumulation of electrolytes against concentration gradients. The theory is seemingly amenable to generalization and extension and can be applied to systems which show a greater similarity to living cells than those considered in the 1955 paper. (Sollner.)

The kinetics of the self-exchange of ions across permselective membranes (the simplest conceivable case of electrolyte kinetics across membranes) was studied by means of radioactive tracers. With the majority of types of membranes there is a reasonable quantitative agreement between the rate of self-exchange and a rate which can be computed on the basis of simple assumptions from the electrical resistance of the membrane

in the same solution. (With the before-mentioned poly-2-vinyl-N-methyl pyridinium membrane and some other strong-base membranes this relationship, for unknown causes, does not hold true.) The self-exchange studies are primarily a precursor to the study of allo-exchange across permselective membranes, that is of the mutual exchange of two different species of ions of the same charge. Hopeful leads have been obtained to the theoretical treatment of this phenomena which aims at the prediction of absolute flux rates from purely electrical data. (Gottlieb and Sollner.)

Significance to NIAMD Research: In order to understand electrolyte relationships in living cells and tissues, it is necessary to have accurate information on membrane model systems which, under carefully controlled known conditions, reproduce at least some of the major *in vivo* phenomena. The work of recent years, particularly the study of polyionic potentials and the construction of an *in vitro* model of electrolyte accumulation, together with the before-described effects on flux rates in related phenomena have brought us significantly nearer to an understanding and an *in vitro* reproduction of the type of effects which ultimately must govern the *in vivo* osmotic behavior of cells and tissues. The work already carried out seems to indicate that even fairly complex membrane systems, similar to those found in living nature, may prove in the foreseeable future, amenable to a complete and quantitative physicochemical analysis.

Proposed Course of the Project: Continuation of the theoretical work on electrolyte accumulation (Sollner). Construction and exploration of experimental model systems to study in some detail electrolyte accumulation (Lewis and Sollner). Preparation of improved permselective protamine collodion membranes (Gottlieb, Lewis and Sollner). Studies on the correlation of diffusion, self-exchange, and resistance in and across permselective membranes (Wagner and Sollner). Continuation of the theoretical and experimental work on the absolute rates of self-exchange and of allo-exchange across permselective membranes (Gottlieb and Sollner). Theoretical and experimental studies on the relative rates of exchange (relative flux rates) of several co-existing species of ions of the same charge across permselective membranes in continuation of the work on polyionic potentials listed in the 1955 list of publications (Gershfeld and Sollner).

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Dr. Melvin Gottlieb, Laboratory of Kidney and Electrolyte Metabolism, National Heart Institute, cooperates on a voluntary basis with the principal investigator on the studies of absolute reaction rates in membrane systems. He also participates in the preparation of improved permselective membranes.

Dr. Joseph Wagner, of Vienna, Austria, assigned through the Institute of International Education to the principal investigator's laboratory for the period January 1st to September 7, 1955 (see above).

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No parallel research.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

The Electrochemistry of Porous Membranes. "Electrochemistry in Biology and Medicine," edited by Theodore Shedlovsky, John Wiley and Sons, New York, 1955, pp. 33-64.

Membranes of High Electrochemical Activity in Studies of Biological Interest. S. Dray, Eugene Grim, Rex Neihof and K. Sollner. "Electrochemistry in Biology and Medicine," edited by Theodore Shedlovsky, John Wiley and Sons, New York, 1955, pp. 65-90.

A Physicochemical Cell Model which Simultaneously Accumulates Anions and Cations against Concentration Gradients. K. Sollner. Arch. Biochem. and Biophys., 54, 129-134 (1955).

A Quantitative Electrochemical Theory of the Electrolyte Permeability of Mosaic Membranes Composed of Selectively Anion-Permeable and Selectively Cation-Permeable Parts, and Its Experimental Verification. II. A Quantitative Test of the Theory in Model Systems which do not Involve the Use of Auxiliary Electrodes. K. Sollner and Rex Neihof. J. Gen. Physiol. 38, 613-622 (1955).

Experimental Studies on Bi-ionic Potentials Across Permselective Membranes. K. Sollner and Sheldon Dray. Biochim. et Biophys. Acta 18 (1955) 341-352.

NIMD-77
SERIAL NO.

A Theory of Dynamic Polyionic Potentials across Membranes of Ideal Ionic Selectivity. K. Sollner and Sheldon Dray. Biochim. et Biophys. Acta (in press).

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

No honors or awards.

Analysis of NIH Program Activities

1. National Institute of Arthritis a nd Metabolic Diseases
INSTITUTE

2. Laboratory of Physical Biology
LABORATORY OR BRANCH

3. Molecular Biophysics
SECTION

4.
LOCATION (IF OTHER THAN BETHESDA)

5. NIAMD-78
SERIAL NO.

6. Investigation of the macromolecular organization of living matter.
PROJECT TITLE

7. Dr. Ralph W. G. Wyckoff
PRINCIPAL INVESTIGATOR(S)

8. Dr. Louis W. Labaw, Mr. Vernon M. Mosley, Mlle. O. Croissant, (Visiting Scientist), Dr. W. C. Nixon (Visiting Scientist), Persis Griffin.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To gain information about the macromolecules that are essential constituents of living matter, to see how they are arranged in the structures they form and to see how this arrangement is altered by infectious and degenerative disease. To study certain of these macromolecules (such as viruses) in purified form after isolation from the living material.

Methods Employed: The electron microscopy of microorganisms, cells, and tissues in suspension or thinly sectioned; the physicochemical characterization of macromolecular components isolated from such material using electron microscopy, X-ray diffraction, and similar established techniques; the development of new physical procedures, including X-ray microscopy, to further such characterization.

9. PROJECT DESCRIPTION - CONTINUED

Major Findings: The work done in Bethesda during the last year has proceeded along the following main lines:

(1) The development of better methods, involving the use of evaporated carbon films, for reproducing macromolecular order existing on the surfaces of crystals and other solids. These methods have been applied to the elucidation of the structures of a number of virus and other protein crystals and, in collaboration with the Institute of Dental Research, to the further study of the fine structure of tooth enamel.

(2) A continuation of the collaborative work with the Pasteur Institute in Paris dealing with the visualization of neurotropic viruses within diseased nervous tissue. During the last year, we have made considerable progress in tracing the development of herpes virus in brain and have succeeded in visualizing the virus of poliomyelitis within cells of the brain. The progress of these investigations was greatly furthered by having Mlle. O. Croissant of the Pasteur Institute here for three months as a Visiting Scientist.

(3) Further improvement in the methods of preparing thin sections for electron microscopic investigation. It is the goal of these studies, which are continuing and are being carried out in collaboration with the Institute for Dental Research, to make the microscope a useful tool for the solution of histological as well as cytological problems. As these methods are being improved, they a re being applied to other aspects of the virus problem, and, in collaboration with the dentists, to studies of the way teeth develop.

(4) A projection X-ray microscope has been put in operation and studies begun to determine its applicability to the problems we have under way. Since this type of instrument is so new that its fields of usefulness are still little known, we have been devoting considerable effort to an exploration of its value in histology and embryology before concentrating on its application to our specific problems. We have, however, begun studies of the calcification of developing and aging tissues and of certain pathological changes induced by virus diseases.

9. PROJECT DESCRIPTION - CONTINUED

Significance to NIAMD Research: Planned as a program of fundamental research, our results may be expected only gradually to be applicable to the specific problems of this Institute. As they take shape, our results should, instead, be useful in studies of pathological conditions in general and of a variety of embryological questions.

Proposed course of project: For the immediate future, the available facilities will be fully employed in continuing the development already outlined with the specific objects of (a) extending still further the use of the electron microscope as a means of observing the macromolecular constituents of healthy and diseased tissues, (b) of exploring the possibilities of the X-ray microscope for the study of biological material, (c) of developing further the application of the electron microscope to histology and, (d) of using these methods in the studies with which this section has been occupied.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 OR 1956:

NO COOPERATING UNITS

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

NO PARALLEL RESEARCH IN PHS

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Croissant, O., Lepine, P., and Wyckoff, Ralph W. G., RECHERCHES SUR L'ULTRASTRUCTURE DES CORPS DE NEGRI EXAMINES AU MICROSCOPE ELECTRONIQUE. Annales de L'Institut Pasteur, 89: 183, 1955.

Croissant, O., Lepine, P., and Wyckoff, Ralph W. G., ETUDE AU MICROSCOPE ELECTRONIQUE DES LESIONS NEVRAXIQUES INTRACELLULAIRES CAUSEES PAR LE VIRUS HERPETIQUE. Annales de L'Institut Pasteur - (In press).

Labaw, L. W., and Mosley, V. M., Periodic Structure in the Flagella of Brucella Bronchiseptica. Biochim. et Biophys. Acta, 17: 322, 1954.

Labaw, L. W. and Wyckoff, Ralph W. G., Molecular Arrangement in Crystals of the Southern Bean Mosaic Virus Protein. Nature, 176: 455, 1955.

Labaw, L. W. and Wyckoff, Ralph W. G., The Crystal Structure of the Turnips Yellows Virus Protein. SCIENCE - (In press).

Labaw, L. W. and Wyckoff, Ralph W. G., The Electron Microscopy of Protein Crystals. Proc. Royal Netherlands Acad. of Sciences - (In press).

Lepine, P., Croissant, O., and Wyckoff, Ralph W. G., ETUDE MORPHOLOGIQUE DE LA CELLULE POLIOMYELITIQUE AU STADE INITIAL DE L'INFECTION. Annales de L'Institut Pasteur - (In press).

Scott, David B. and Wyckoff, Ralph W. G., Carbon surface replicas for electron microscopy and electron diffraction. Proc. Royal Microscopical Society - (In press).

Wyckoff, Ralph W. G., Die Elektronenmikroskopie von Gewebeschnitten. Z. wiss. Mikroskop., 62: (3) 180, 1955.

Wyckoff, Ralph W. G., Introduction to Symposium on Sub-Microscopic Organization of Cytoplasm. (Leiden, The Netherlands - September 1-8, 1954). To appear in Proc. of the VIIIth Congress of Cell Biology, Leiden (1956). (In press).

16. LIST OF PUBLICATIONS - CONTINUED

Wyckoff, Ralph W. G. and Labaw, L. W., On the Structure of Macromolecular Crystals. Exptl. Cell Research, 127: Suppl. 3, 395, 1955. (Special Volume for "RUNNSTROM").

Wyckoff, Ralph W. G. and Labaw, L. W., UTILSATION DES REPLIQUES DE CARBONE POUR ENREGISTRER L'ORDRE MOLECULAIRE DANS LES CRISTAUX MACROMOLECULAIRES. Proceedings of the Colloquium in Toulouse, France, April 4-8, 1955, arranged by French Centre National de la Recherche Scientifique on "Methods on Electron Optics." - (In press).

Young, J. Z. and Wyckoff, Ralph W. G., The Motorneuron Surface. Proc. of The Royal Society - (In press).

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

1. NIAMD — INSTITUTE
2. Laboratory of Physical Biology — LABORATORY OR BRANCH
3. Physical Biochemistry — SECTION
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NIAMD-79 — SERIAL NO.

6. Purification and characterization of the sensitized sheep cell agglutination activity factor and its inhibitory components.
PROJECT TITLE

7. Dr. R. R. Williams
PRINCIPAL INVESTIGATOR(S)

8. S. S. Stone, R. L. Evans, and D. Francois
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The sensitized sheep cell agglutination gives promise of becoming a useful diagnostic procedure in rheumatoid arthritis. Purification and identification of the components of the system including inhibitors promises further insight into the causative agents and pathogenesis of the disease as well as furnishing a more precise diagnostic test.

Methods Employed: The problem involves the use of two biological assay methods: (1) the sensitized sheep cell agglutination for the agglutinator, (2) a semi-quantitative inhibition test developed by the group which yields relative inhibitory activity in terms of concentration of substance tested. Electrophoretic methods have been used for separation and analysis of active fractions. Paper chromatography and ultraviolet spectroscopy have yielded data on amino acids, carbohydrates, and heterocyclic components in active inhibitory preparations. Some of these are free, others obtained by hydrolysis of active protein preparations.

9. PROJECT DESCRIPTION - CONTINUED

Active preparations were obtained by S. S. Stone using tertiary butanol, formic acid, and water mixtures. Binding and equilibrium methods were developed by R. R. Williams. Synthesis of active compounds of known structure and closely related inactive analogues and homologues was performed by R. L. Evans.

Major Findings: The properties of the inhibitor have received most attention. By electrophoretic separation, binding studies, and variation in activity it has been demonstrated that only a small part of Cohn's Fraction II shows inhibitory activity. Equilibrium between inhibitor bound to agglutinator and the free form reveal rate and equilibrium properties which may yield more quantitative data than agglutination assays. Structural studies aimed at defining the chemical specificity of the reaction have shown that glycyl glycine, ureido-succinic acid, 5-acetic acid hydantoin and glucosamine show some inhibitory activity. It is postulated that these compounds or their metabolic products may act by competitive binding to one or another component in the system.

Significance to NIAMD research: The program relates to understanding of the nature and pathogenesis of rheumatoid arthritis.

Proposed Course of Project: Further elaboration of the above studies in the direction of identification and purification of enzymes, antibodies or haptenic groups involved are planned. Also, further binding studies between purines or their intermediary metabolites and the proteins involved in the agglutination are projected. The development of quantitative assay methods is of prime importance.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Collaboration in clinical studies is continuing with Drs. J. J. Bunim, K. L. Yielding and R. L. Black of the Arthritis and Rheumatism Branch of Clinical Investigations.

Collaboration in clinical studies with medical staff members at Mt. Alto V.A. Hospital and Walter Reed Army Hospital.

NIAMD-79
SERIAL NO.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No parallel research in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Bunim, J. J., Sokoloff, L., Williams, R. R., and Black, R. L. Rheumatoid Arthritis: A review of recent advances in our knowledge concerning pathology, diagnosis, and treatment. J. of Chronic Diseases, 1: 168, (1955).

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

1. NIAMD (INSTITUTE) 2. Laboratory of Physical Biology (LABORATORY OR BRANCH)

3. Physical Biochemistry (SECTION) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NIAMD-80 (SERIAL NO.)

6. The mechanism of the orderly aggregation of proteins in building up biological structures.
PROJECT TITLE

7. Dr. Koloman Laki and Dr. H. A. Saroff
PRINCIPAL INVESTIGATORS

8. Dr. W. J. Bowen, Dr. F. Irreverre, A. R. Hayden, Dr. D. R. Kominz, E. R. Adamik, T. D. Kerwin, Dr. W. R. Carroll, M. J. Callanan, Farida M. Saad, Dr. R. B. Simpson, E. R. Mitchell, Dr. E. Mihalyi, Visiting Scientist
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objective of this project is to study the mechanism of polymerization of proteins in order to gain insight as to how cells build up network structures. The structures involved in muscular contraction and blood coagulation are the results of protein polymerization. The main part of this project is, therefore, devoted to the study of proteins involved in muscular contraction and blood coagulation.

Methods Employed: The method of attack is both direct and indirect. In the direct attack the proteins of muscular contraction and blood coagulation are separated from their native milieu and are studied under arbitrary conditions which are selected to reveal properties of interest. (Isolation; characterization: size and shape of the molecules; determination of chemical composition; modifications brought about by changing specific

9. PROJECT DESCRIPTION - CONTINUED

groupings either chemically or enzymatically.) In the indirect attack studies are made on some other already better known proteins to gain information before the direct attack is made. In these studies the procedures of biochemistry and physico-chemistry are employed. For example: paper and ion-exchange chromatography, enzymology, ultracentrifugal analysis, osmometry, light-scattering measurements, electrophoresis, the diffusion measurements, etc.

Major Findings: The contraction of muscle fibers in KSCN and HgI_2.KI solutions was found to be a temperature dependent equilibrium reaction (Laki, Bowen).

The determination of amino acid composition of the contractile muscle proteins: myosin and tropomyosin, was extended to different muscle tissues of various animals. Tropomyosin was isolated and crystallized from pregnant human uterus, rabbit skeletal and uterus muscle, calf heart, frog muscle, lobster muscle, earthworm, and flatworm. The amino acid composition exhibited an increasing degree of randomness going from the higher developed animals to the lower ones. This gradual change in amino acid composition is reflected in the total overall electric charge of the molecules (difference in electrophoretic mobility) (Kominz, Saad, Laki).

Amino acid composition of lobster myosin was also found to exhibit increased randomness (Kominz, Saad).

Myosin from pregnant and nonpregnant human uteri was found to behave similarly to myosins from skeletal muscle (Kominz in cooperation with A. Csapo).

Rabbit myosin was found to contain a high molecular weight ribonucleic acid closely associated with it, the removal of which, however, left the physical-chemical and enzymatic properties of myosin unchanged (Mihalyi, Laki).

The main intracellular cation, potassium, was found to be bound to carboxylic groups of myosin (Lewis, Saroff, Bowen).

Amino acid composition of protomyosin preparations (fragment of myosin) was carried out and found to be in agreement with predicted values (Laki, Kominz, Saad, Szent-Györgyi).

9. PROJECT DESCRIPTION - CONTINUED

The enzyme, myokinase, was obtained in a high state of purity (Bowen, Kerwin).

As a significant achievement, a new tropomyosin (to be called "sulfhydro-tropomyosin") containing free SH-groups was isolated from rabbit muscle (Kominz).

The determination of SH-groups in serum albumin under various conditions was undertaken in order to discover whether the varying amount of SH-groups found in it can be accounted for by a reversible thiazoline ring formation (Saroff, Simpson).

The guanidination of proteins, successfully used previously for serum albumin and fibrinogen, was extended to insulin (Saroff, Irreverre, Adamik).

A quantitative Sakaguchi reaction was developed to deal with arginine and the guanidinated amino acids (Irreverre, Kominz).

The binding of sulfate and phosphate ions to the protein salmine was studied (Carroll, Callanan, Mitchell).

An improved method of paper chromatography was devised for the separation of the diastereoisomers of cylic hydroxy-amino acids (Irreverre) and successfully used for the identification of 5-hydroxy pipecolic acid and its diastereoisomer from various natural sources (Irreverre in cooperation with B. Witkop, L. A. Cohen).

Bovine fibrinogen was further purified and crystallized, and used as a test material for the quantitative determination of the Laki-Lorand factor found to be missing from the plasma in some cases of the disorders of bone marrow (Laki).

The heat burst during clotting of purified fibrinogen has been determined under various conditions using the Benzinger micro calorimeter (Laki in cooperation with H. Benzinger).

9. PROJECT DESCRIPTION - CONTINUED

Significance to NIAMD Research: When part of the protoplasm or the whole cell (as in, e.g., cell division or muscular contraction) performs mechanical work, a network structure is built up at least temporarily, mainly through an orderly polymerization of globular proteins. This structure then reacts with the surrounding material and by utilizing metabolic energy (stored in ATP e.g.) performs work (muscular contraction, ameoboid movement). In order to understand this "mechano-chemical coupling" (the interaction of structure with the surrounding) and its disorders, we must know how such structures are built up. Muscular contraction and blood coagulation are examples of processes where structures are built up through protein polymerization. Such knowledge eventually will lead us to the understanding of certain diseases of muscle. Study of blood clotting, in addition to supplying clues for protein polymerization, gives us better understanding of the disorders of blood clotting (like hemophilia and thrombus formation).

When both direct and indirect approach leads to some specific disease (e.g. hemophilia, rheumatoid arthritis) the advantage offered by studying the disease is utilized to the extent profitable.

Proposed Course of Project: In the next calendar year the project will be pursued along the lines presented above. The partial analysis of the amino acid sequence of one of the peptides of co-fibrin is in progress together with the determination of the carboxylic end groups of fibrinogen and fibrin. The purification of the L-L factor is planned. The usefulness of O-methylisourea as an end-group reagent will be further investigated on the hormone: insulin.

NIAMD-80
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Dr. B. Witkop and Dr. L. A. Cohen, Laboratory of Chemistry, NIAMD (cooperating with Irreverre).

Dr. A. G. Szent-Györgyi, Institute for Muscle Research, Woods Hole, Mass. (cooperating with Laki, Kominz).

Dr. A. Csapo, Department of Embryology, Carnegie Institution, Baltimore, Md. (cooperating with Laki and Kominz).

Dr. H. Benzinger, Naval Medical Research Institute (cooperating with Laki).

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTER-CHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

No parallel research in the PHS.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Bowen, W.J., and Laki, K.: The contraction of muscle fiber and myosin B thread in KI and KSCN solutions. Biochim. et Biophys. Acta, 16: 301, Feb., 1955.

Bowen, W. J., and Laki, K.: The effect of the concentration of KI and of temperature on the shortening of glycerol-treated muscle fibers in KI solutions. Am. J. Physiol., in press.

Bowen, W. J., and Kerwin, T. D.: The rate of dephosphorylation of adenosinetriphosphate and the shortening of glycerol-washed muscle fibers. Biochim. et Biophys. Acta, 18: 83-86, Sept., 1955.

Bowen, W. J., and Kerwin, T. D.: Effect of uranyl chloride upon adenosinetriphosphatase of myosin. Proc. Soc. Exptl. Biol. and Med., 88: (4), 515-517, Apr. 1955.

Bowen, W. J., and Kerwin, T. D.: A simple method for the analysis of ATP and ADP in mixtures. J. Biol. Chem., in press.

Laki, K., and Carroll, W. R.: Size of the myosin molecule. Nature, 175: 389, Feb. 26, 1955.

Bowen, W. J., and Kerwin, T. D.: The purification of myokinase with ion exchange resin. Arch. Biochem. and Biophys. 57: (2) 522-524, Aug. 1955.

Spicer, S. S., and Weise, V. K.: Studies on myosin aggregation. Arch. Biochem. and Biophys. 59: (2) 313-325, Dec., 1955.

Saroff, H. A., Loeb, G. I., and Scheraga, H. A.: Low and high pH effects on serum albumin. J. Am. Chem. Soc., 77: 2908 (1955).

Callanan, M. J., Carroll, W. R., and Mitchell, E. R.: The binding of sulfate and phosphate ions by salmine. Biochim. et Biophys. Acta, 18: 462-463 (1955).

Saroff, H. A.: An easily assembled continuous electrophoresis apparatus. Nature, 175: 896, May 21, 1955.

SERIAL NO. NIAMD-80

16. PUBLICATIONS - CONTINUED

Adamik. E.: Modification of continuous electrophoresis apparatus. Analytical Chemistry, in press.

Simpson, R. B., Evans, R. L., and Saroff, H. A.: The polarography of the noble metals. J. Am. Chem. Soc. 77: 1438 (1955).

Saroff, H. A.: Polarographic Techniques. Louis Meites. Science, 122: (3171), 1094, Dec. 2, 1955. Book review.

Cohen, L. A., Irreverre, F., Piez, K. A., Witkop, B., and Wolf, H. L.: Synthesis of 5-hydroxy pipecolic acid and separation of its diastereoisomers. Science, in press.

Lewis, M. S., Studies of binding of potassium and sodium to the contractile proteins of muscle. Ph.D. Thesis. Georgetown University, 1955.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

K. Laki has been elected a member of Washington Academy of Sciences.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Pathology and Histochemistry 3. Histochemistry
LABORATORY SECTION

4. Bethesda 5. NIAMD-81
LOCATION SERIAL NUMBER

6. Development and exploration of histochemical reactions in tissues
PROJECT TITLE

7. R. D. Lillie
PRINCIPAL INVESTIGATOR

8. J. B. Longley, R. C. Bahn, G. Glenner, S. Spicer
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To further correlate chemical and morphologic structure of normal and diseased tissues by devising or adapting chemical reactions to histologic study and to explore and interpret existing chemical and empirical methods.

(Underlined names are those of members of Histochemical Section.)

a. Functioning transplantable mouse pituitary tumors are better classified by bioassay and transplantability than histochemically. Melanophore stimulating hormone has been isolated and assayed and requires O_2, ATP and glucose phosphate for action. MSH tumors produce a host syndrome of adrenal hypertrophy, water diabetes and cessation of estrus cycle, and responsible steroids are isolated from blood and urine. Bahn, Condliffe, Wilson, Furth et al.

b. Isolated prolactin and pituitary powders produce in pigeon crop epithelial hypertrophy and pronounced basophilia, urine from lactating women does not. Bahn and Bates.

c. By zone microdissection and statistical analysis of tubule zone prevalence, succinic dehydrogenase has been quantitated by renal tubule segments. Blue tetrazolium produces mono and diformazans, the latter has much higher color density per mole. More accurate quantitation is done with iodotetrazolium monoformazan. Therapeutic mercurial diuresis does not depend on measurable impairment of succinic dehydrogenase. Longley and Bahn.

d. A brief, quite selective, but rather insensitive adaptation of the Ehrlich pyrrole reaction to histochemistry demonstrates strong reactivity of lens protein and pancreatic zymogen, weaker of pepsinogen, dubious of melanin and none in enterochromaffin. Lillie.

e. Gastric and intestinal enterochromaffin may differ histochemically in the same species as well as from one species to another. Model experiments with 5-OH- and plain tryptamine, compared with enterochromaffins, suggest non-identity.

f. The yellow pigment of guinea pig hair and the nerve cell melanin of man appear to differ from other melanins in the same species histochemically, and there appear to be also species differences in melanins of corresponding sites.

g. Dark blue Nile blue staining of fatty acid lipofuscins appears to depend partly on an oil solubility and indicator property of the dye rather than purely on dye salt formation. Lillie.

h. Spinal cord lipofuscin gives direct aldehyde reactions despite primary fixation in hydroquinone formalin, contra Karnowsky and Deane for adrenal lipid, and resists primary fixation in hot methanol chloroform. Lillie.

i. Studies on histochemical adaptation of tetraporphin reactions have been started (Glenner) and a combined biochemical, histologic, histochemical and electron-micrographic study of the cataractous lens in experimental diabetes is under way (Stetten, Glenner, Wyckoff).

j. Studies on mechanism of elastin staining and of metal hematoxylin staining are being further pursued (Fullmer, Lillie). Dr. Spicer is completing a year's study at the A.F.I.P. in June 1956 and gathering material from certain unusual conditions for future histochemical study.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-81
SERIAL NO.

12.
BUDGET ACTIVITY: Research

13.
IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

NIAMD: Section Endocrinology - LBN: Dr. E. Anderson

Section on Molecular Biophysics - LPB: Dr. Wyckoff

Section on Intermediary Metabolism - LBN : Dr. D. Stetten Jr.

NIDR: Section on Clinical Investigation, SERIAL NO:_____ : Dr. H. M. Fullmer

NCI: Section on Endocrinology, SERIAL NO:_____ : Dr. S. Fand

14.
NO PARALLEL RESEARCH IN THE PHS.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-81
SERIAL NO.

16.
LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Lillie, R. D., and Henson, J. P. Greco: Xylene-cellulose caprate as a histologic mounting medium of low refractive index. Stain Tech. 30: 133-134, 1954.

Longley, J. B., and Bahn, R. C.: Problems in quantitating renal enzymes. J. Histochem. and Cytochem. 3: 274-276, 1955.

Longley, J. B.: Alkaline phosphatase in the kidneys of aglomerular fish. Science. 122: 594, 1955.

Monis, B., and Longley, J. B.: Mucins in the epithelim of renal collecting tubules. Nature. 176: 741-742, 1955.

R. D. Lillie: The ba sophilia of melanins. J. Histochem. and Cytochem. 3: 453-454, 1955.

Fullmer, H. M., and Lillie, R. D.: Some aspects of the mechanism of orcein staining. J. Histochem. and Cytochem. 4: 64-68, 1956.

G. C. Zorzoli and Lillie, R. D.: Ricerche istochimiche sulle cellule "enterocromaffini". In press.

G. C. Zorzoli: Ricerche istochimiche sul pigmento delle cellule nervose del "Thalamus opticus" di uomo. In press.

Lillie, R. D.: The p-dimethylaminobenzaldehyde reaction for pyrroles in histochemistry: melanins, enterochromaffin, zymogen granules, lens. J. Histochem. Cytochem. 4: ---, 1956. In press.

Lillie, R. D.: Phenolic oxidative activities of the skin - Some reactions of keratohyalin and trichohyalin. J. Histochem. and Cytochem. 4: ---, 1956. In press.

Lillie, R. D.: The periodic acid Schiff reaction in pathology. Am. J. Clin. Path. --- (editorial) In press.

16. LIST OF PUBLICATIONS (Cont'd)

Longley, J. B. : Histochemical or histological techniques? Bull. N. Y. Acad. Med. 32:83-85, 1956.

Longley, J. B., and Fisher, E. R.: A histochemical basis for changes in renal tubular function in young mice. Quart. J. Micr. Sci. In press.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR

None

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases (INSTITUTE) 2. Pathology and Histochemistry (LABORATORY)

3. Hematology (SECTION) 4. _____ (LOCATION) 5. NIAMD-82 (SERIAL NO.)

6. Studies of liver cell mitochondria
PROJECT TITLE

7. George L. Fite
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: To determine relations of mitochondrial changes to metabolic processes in the liver.

Major Findings:

1. It has been found that abnormal mitochondria appears as an early change in dietary liver cell degeneration. These may undergo dissolution, or, under certain circumstances, calcification. In many or most cases these cells die, but in other cases, cells may survive with mitochondria totally lost. In such cases, the liver cells still retaining mitochondria show numerous fine filamentous forms suggesting that they compensate for cells which have lost mitochondria.

2. There appears to be an anatomic relation between mitochondria and the glycogen metabolism of liver cells. Regeneration of mitochondria appears dependent upon the presence of glycogen.

Significance to NIAMD Research: This work is a basic investigation into the nature of liver cell metabolism, as viewed histologically, and has potentially broad applications in the field.

Future Course of the Project: Much more control in formation is needed to correlate mitochondrial activity with dietary changes. There are undoubted relations to substances added to basic diets and to deficiencies in diets. In addition, further studies are intended to be correlated with hemolysis with the enzyme system operating within the cell.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-82
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. No cooperating units of the Public Health Service or other organizations.

14. No parallel research in the Public Health Service.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases — INSTITUTE
2. Pathology and Histochemistry — LABORATORY
3. Hematology — SECTION
4. _____ — LOCATION
5. NIAMD-83 — SERIAL NO.
6. Pathogenesis of experimental arthritis and pathology of rheumatism — PROJECT TITLE
7. Leon Sokoloff — PRINCIPAL INVESTIGATOR(S)
8. None — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION:

Objectives: Investigation of factors influencing development of degenerative joint diseases and of non-infectious inflammatory arthritis in small laboratory animals. Study of the histopathology of the synovial membrane in human arthritis.

Methods: Histologic and x-ray examinations of small laboratory animals have been used to establish the spontaneous occurrence of degenerative joint disease (osteoarthritis) in aged laboratory animals, some of which have been subjected to experimentally altered environmental factors. The "skeletal age" of these animals has been carefully measured by charting the times of closure of the epiphyses. Multiple inbred strains of mice are used to establish marked differences in the spontaneous incidence of degenerative joint disease and in attempts to characterize the factors influencing the development of osteoarthritis. Trypan blue was injected intraperitoneally into rats in an attempt to produce inflammatory joint diseases. Sensitization to synovial and splenic tissues with Freund's adjuvant, adrenalectomy, DOCA, adrenal vein ligation and parabiosis with sodium chloride feeding will be used to produce non-infectious inflammatory arthritis in rats.

9. (cont'd.)

Major Findings: Existence of osteoarthritis of mice closely simulating human degenerative joint disease has been established. The incidence varied in different strains of mice from a low of 2% to a high of 93%. No correlation of incidence of arthritis with skeletal aging as measured by epiphyseal closure exists.

Significance to NIAMD Research: Degenerative joint disease is the commonest form of arthritis. It entails chronic disability and huge economic losses, thus presenting a public health problem of the first order. Marked variation of the incidence of this type of joint disease in different strains of mice suggests that the disorder is not necessarily an unavoidable accompaniment of old age. Study of the difference in incidence of osteoarthritis in mice offers the hope that factors leading to the development of this disease can be identified, and preventive measures developed that may be applicable to human disease.

Rheumatoid arthritis and certain of the rheumatic diseases are characterized by apparently non-infectious chronically progressive inflammation of joints. The present search for a counterpart of this disease in animals, if successful, will provide the means of studying not only the pathogenesis but also suitable treatment of this chronic and disabling disorder.

Future Course of the Project: Among the factors influencing the degenerative joint disease in mice, nutritional factors will be studied in corporation with Dr. Olaf Mickelsen. Thyroid histology and function will be studied with Dr. J. Rall to determine the role of the thyroid in epiphyseal aging and osteoarthritis. Measurements of spontaneous activity and its relationship to joint disease will be made in the various strains of mice. Genetic studies will be carried out. The effect of selenium will be studied in view of the occurrence of osteoarthritis in selenium poisoning in cattle and horses. The procedures enumerated under methods will be used in an attempt to produce the counterpart of rheumatoid arthritis in laboratory animals. The possible relationship of such lesions to Streptobacilli, which are found frequently in rats, will be studied in collaboration with Dr. Lerner.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-83
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

Studies on tissue antibodies in rheumatoid fever with special reference to the streptococcus (in collaboration with Dr. E. M. Lerner of NIAMD (), and R. M. Cole, NMI ().

Histopathology of the synovial membrane in arthritis will be carried out in collaboration with the Laboratory of Clinical Investigations.

14. No parallel research in the Public Health Service.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-83
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955.

1. Sokoloff, Leon, Bunim, J. J., Williams, R. R., and Black, R.L.: Rheumatoid Arthritis: A Review of Recent Advances in our Knowledge Concerning Pathology, Diagnosis and Treatment. J. Chron. Dis., 1:168-210, 1955.

2. Sokoloff, L.: Tables for Handbook of Biological Data. Chemical Composition of Normal Synovial Fluid. Cytology of Normal Synovial Fluid. In press.

3. Sokoloff, L.: Some Aspects of the Pathology of Collagen Diseases. Proc. N.Y. Path. Soc., Bull. N.Y. Acad. Med. In press.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases — INSTITUTE
2. Pathology and Histochemistry — LABORATORY OR BRANCH
3. Hematology — SECTION
4. _____ — LOCATION
5. NIAMD-84 — SERIAL NO.
6. Studies on abnormal human hemoglobins — PROJECT TITLE
7. H. A. Itano — PRINCIPAL INVESTIGATOR(S)
8. None — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION:

Objectives: To study the physical chemistry, biochemical genetics, and clinical significance of the abnormal human hemoglobins.

a. The simultaneous presence of hemoglobins S and D results in an anemia similar to sickle cell anemia; the simultaneous presence of hemoglobin E and Cooley's trait results in an anemia similar to Cooley's anemia. Sturgeon, Itano, and Bergren.

b. The abnormal hemoglobins are involved in some cases of moderately severe Cooley's anemia. In other cases, only normal adult and fetal hemoglobins are present. Sturgeon, Itano, and Bergren.

c. An abnormal hemoglobin with an isoelectric point lower than that of normal adult hemoglobin has been discovered. Its presence is not associated with anemias. Thorup, Itano, et al.

d. The mobilities of all of the known human hemoglobins, normal and abnormal, have been determined in a series of univalent buffers. These determinations will be used as reference standards to check reports of new hemoglobins. Itano.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-84
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

NIH: Laboratory of Kidney and Electrolyte Metabolism - H. Keitel.

Children's Hospital, Los Angeles, California - P. Sturgeon and W. Bergren.

University of Virginia School of Medicine - O. Thorup

14. No parallel research in the public health service.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-84
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Sturgeon, P., Itano, H. A., and Bergren, W. R.: Clinical Manifestations of Inherited Abnormal Hemoglobins. 1. The Interaction of Hemoglobin-S with Hemoglobin-D. 2. Interaction of Hemoglobin-E and Thalassemia Trait, Blood, 10:389-404, 1955.

Sturgeon, P., Itano, H. A., and Bergren, W. R.: Genetic and Biochemical Studies of "intermediate" Types of Cooley's anemia, Brit. J. Hematology, 1:264-277, 1955.

Itano, H. A.: Clinical States Associated with Alterations of the Hemoglobin Molecule, A.M.A. Arch. Int. Med., 96:287-297, 1955.

Itano, H. A.: The Hemoglobins, Annual Rev. Biochem. In press.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Delivered the Fifth George Minot Lecture before the Section on Experimental Medicine and Therapeutics, American Medical Association, June 8, 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases — INSTITUTE
2. Pathology and Histochemistry — LABORATORY
3. Hematology — SECTION
4. _____ — LOCATION
5. NIAMD-85 — SERIAL NO.
6. Physiology of white cells in diabetes — PROJECT TITLE
7. Jane Shohl and George Brecher — PRINCIPAL INVESTIGATOR(S)
8. None — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION:

Objectives: Characterization of the metabolic abnormality of white cells in diabetes and the possible relationship to the susceptibility to infections. As employed, diabetes will be produced experimentally in rabbits by alloxan. The metabolism of the white cells will be studied using chemical and tracer methods. The role of demonstrable abnormalities of white cells in the increased susceptibility to infection of diabetic animals and men will be studied.

Major Findings: This project was initiated one month ago and there are no major findings.

Significance to NIAMD Research: The susceptibility of diabetics to infection is one of the unsolved puzzles of this important disease.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-85
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. No cooperating units of the Public Health Service or other organizations.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

This project complements investigations by Dr. Gorsuch (), Laboratory of Clinical Investigations, National Institute of Microbiology, whose studies will be concerned primarily with the clearing mechanism of bacteria in diabetics rather than with the specific function of white blood cells.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-85
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases — INSTITUTE
2. Pathology and Histochemistry — LABORATORY
3. Hematology — SECTION
4. _____ — LOCATION
5. NIAMD-86 — SERIAL NO.
6. Regulation of hemopoiesis — PROJECT TITLE
7. Frederick Stohlman, Jr., and George Brecher — PRINCIPAL INVESTIGATOR(S)
8. None — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION:

Objectives: The identification and characterization of substances capable of stimulating red cell production.

Methods Employed: Substances capable of stimulating red cell production are assayed by injecting them into irradiated rats which respond better than normal animals to such stimulation. The response is measured by the incorporation of radioactive iron into red cells. It has been shown that under the conditions used this is an accurate measure of red cell production. Other methods employed for the measurement of red cell production include the clearance of radio-iron, and survival studies of red blood cells using radio-chromium as a tracer.

Major Findings:

1. The presence of a substance capable of stimulating production of red blood cells has been demonstrated in the plasma of anemic rats. This substance is stable at room temperature for several days, and stable at ice box temperature for several weeks. It is unstable at body temperature.

2. A similar or identical stimulating substance is present in the blood of animals exposed to altitude.

9. Major Findings (cont'd.)

3. The oxygen lack induced by exposure to altitude appears to have a dual effect. On the one hand, it appears to stimulate red cell production by elaboration of a factor which stimulates the bone marrow. On the other hand, oxygen lack of the bone marrow itself inhibits erythropoiesis. Consequently, maximal response to the stimulus of hypoxia is not always obtained on exposure to altitude because of these conflicting influences of hypoxia.

4. Early damage to red cells following whole body irradiation has been demonstrated. This very subtle damage becomes apparent only when a second noxious influence acts on the minimally damaged red cells.

Significance to NIAMD Research: Anemia is a common complication of arthritis and may be refractory to treatment. A better understanding of regulation of erythropoiesis should result eventually in improved therapy.

Proposed Course of Project: Physical-chemical means will be employed for fractionation of the plasma and the various plasma fractions will be tested for the presence of erythropoietic factor. Concentration of this stimulator will also be attempted.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-86
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

Dr. S. J. Sarnoff (), Laboratory of Cardiovascular Hemodynamics and Dr. F. Stohlman, Jr. () are collaborating in the study of anemia produced by the mechanical damage to red cells in experimental animals and men carring plastic heart prosthesis.

14. No parallel research in the Public Health Service.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-86
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955.

1. Stohlman, F., Jr., Cronkite, E. P., and Brecher, G.: Stimulation of Erythropoiesis in Irradiated Dogs and Rats. Proc. Soc. Exp. Biol. & Med., 88:402, 1955.

2. Stohlman, F., Jr.: Red Cell Survival in the Dog Determined by a Method of Differential Agglutination Employing Canine Anti-A Serum. J. of Lab. & Clin. Med. January, 1956.

3. Stohlman, F., Jr., and Schneiderman, M.: Application of the Cr^{51} Technic to the Study of Experimental Hemolysis in the Dog. J. of Lab. & Clin. Med., January, 1956.

4. Stohlman, F., Jr., Sarnoff, S. J., Case, R. B., and Ness, A. T.: Hemolytic Syndrome Following the Insertion of a Lucite Ball Valve Prosthesis Into the Cardiovascular System. Circulation. In press.

5. Cronkite, E. P., and Brecher, G.: Sort des Leucocytes Transfusés dans les Chiens Atteints d'Aplasie de la Moelle Osseuse. Le Sang, 26:260, 1955.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases — INSTITUTE
2. Pathology and Histochemistry — LABORATORY
3. Pathologic Anatomy — SECTION
4. _____ — LOCATION
5. NIAMD-87 — SERIAL NO.
6. Project A: Production of Experimental bacterial endocarditis and glomerulonephritis.
 Project B: High Altitude.
 Project C: Toxicity of Iodides and Iodates.
 Project D: Pathology of trypanosomiasis in rabbits.
 PROJECT TITLE
7. B. Highman.
 PRINCIPAL INVESTIGATOR
8. P. D. Altland (Projects A and B); J. Roshe (Project A); S. E. Webster (Project C); E. J. Tobie (Project D).
 OTHER INVESTIGATORS
9. PROJECT DESCRIPTION:

Project A - Production of Experimental bacterial endocarditis and glomerulonephritis.

Objectives: To produce endocarditis and glomerulonephritis, to determine predisposing factors, to study the development of the lesions and causes of treatment failures, and to test in vivo the effectiveness of various forms of therapy.

Methods Employed: Aortic insufficiency is induced in dogs by perforating an aortic leaflet with punch introduced through an incision in the ascending aorta. Beginning about two weeks after surgery, five-hour bacterial broth cultures, 1 or 2 cc. per dose, are injected intravenously.

Major Findings: Endocarditis resembling acute and subacute bacterial endocarditis in man was produced consistently by Staphylococcus aureus and Streptococcus mitis respectively; a single injection was effective. Dogs given staphylococci usually died in a week while dogs given S. mitis were sacrificed in 10 to 14 days. The valvular lesions in both groups were confined largely to the contact surfaces of the mitral and aortic leaflets and to the

9. (Cont'd)

endocardium facing the perforation in the aortic leaflet; these are the areas of greatest hemodynamic stress in aortic insufficiency. Dogs given S. mitis often showed a diffuse proliferative glomerulonephritis similar to that associated with human endocarditis. A Hufnagel valve, inserted below the arch of the aorta before bacteria were injected, afforded no striking protection against endocarditis.

Significance: Our findings indicate that dogs with aortic insufficiency are useful in the study and production of experimental endocarditis and glomerulonephritis and may aid in achieving the objectives outlined above. The failure of the Hufnagel valve, which reduces cardiac work load, suggests that additional factors, perhaps a deficient coronary and valvular blood flow, play a role in susceptibility to endocarditis. An increased work load has been shown to be a factor in rendering altitude animals, described in Project B, susceptible to endocarditis.

Proposed Course of Project: Studies will be made on the natural course of the untreated infection in dogs given S. mitis, on the effects of antibiotic therapy, and on the susceptibility of dogs with aortic insufficiency to other bacteria.

Project B - High Altitude Studies.

Objectives: To study effects of discontinuous exposure to simulated high altitude (hypoxia) on various animals.

Methods Employed: Animals are exposed several hours daily to a simulated altitude of 25,000 feet.

Major Findings: Damage to the heart and other organs is less in dogs than in similarly exposed rats. Dogs develop moderate susceptibility to endocarditis, but this is less than in similarly exposed rats and less than in dogs with aortic insufficiency described in Project A.

Significance: Findings may be significant to those concerned with aviation medicine, health of high altitude inhabitants, and laboratory investigation of cardiorenal, endocrine and other metabolic disorders.

Proposed Course of Project: In addition to continuing above, studies will be made on obese rats to determine influence of high fat diets on the development of pathologic lesions attributable to high altitude.

9. (Cont'd)

Project C - Toxicity of Iodides and Iodates

Objectives: To determine possible health hazards in use of iodides and iodates, particularly for prevention of colloid goiter.

Methods Employed: Animals are examined histologically at various periods after administration of iodides and iodates by various routes.

Major Findings: Iodates cause damage to parietal cells of stomach and to retina.

Significance: May be useful tool in studying pathogenesis of certain eye and stomach disorders.

Proposed Course of Project: Studies will be continued, using dogs, to determine safe therapeutic limits.

Project D - Pathology of Trypanosomiasis in Rabbits.

Objectives: To produce lesions in rabbits comparable with the lesions in the nervous system found in late stages of human trypanosomiasis (African sleeping sickness).

Methods Employed: Animals inoculated with trypanosomes are treated after successive relapses (with aminonucleoside of puromycin) to prolong life sufficiently for the late stages of the disease to develop.

Major Findings: Central nervous system lesions comparable with those of human trypanosomiasis have been produced in infected mice so treated.

Significance: May be useful in studying pathogenesis and treatment of African sleeping sickness.

Proposed Course of Project: Depends on results.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-87
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

Laboratory of Physical Biology, Section on Physiology, NIAMD (Project A and B).

Clinic of Surgery, National Heart Institute (Project A).

Laboratory of Pharmacology and Toxicology, Section on Pharmacolgy, NIAMD (Project C).

Laboratory of Infectious Diseases, National Microbiological Institute (Project D).

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO(S). IF WITHIN NIH).

No parallel research in the Public Health Service.

Analysis of NIH Program Activities

Honors, Awards, and Publication Sheet

15. NIAMD-87
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Highman, B. and Altland, P. D.: Effect of altitude and cobalt polycythemia, hypoxia, and cortisone on susceptibility of rats to endocarditis. Circulation Research 3:351-356, 1955.

Tobie, E. J., and Highman, B.: Influence of the amino nucleoside of puromycin on the course and pathology of trypanosome infections in rabbits and mice. Am. J. Trop. Med. and Hyg. In press.

Highman, B., Roshe, J. and Altland, P. D.: Production of endocarditis with Staphylococcus aureus and Streptococcus mitis in dogs with aortic insufficiency. Circulation Research. In press.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases (INSTITUTE) 2. Pathology and Histochemistry (LABORATORY)

3. Pathologic Anatomy (SECTION) 4. _____ (LOCATION) 5. NIAMD-88 (SERIAL NO.)

6. Study of Mechanisms Involved in Infectious Diseases (PROJECT TITLE)

7. E. M. Lerner II (PRINCIPAL INVESTIGATOR(S))

8. Elizabeth Verder, Alexis Shelokov, Roger Cole, and Leon Sokoloff (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION:

Objectives: The objective of this project is to study the course of natural infections in man, and natural and artificially induced infections in animals, employing bacteriological, pathological, immunological, and chemical methods of investigation, in order to elucidate some of the factors influencing host-parasite relationship.

A. Streptobacillus moniliformis Infections.

B. Tissue Antibodies in Rheumatic Fever with Special Reference to the Streptococcus.

Methods Employed: A. - Animals have been injected and studied culturally and histologically to follow the pathogenesis of Streptobacillus moniliformis infection. Specialized cultural methods have been employed to recover this microorganism in cases of atypical human infections. Immunological techniques have been applied to the identification of this microorganism.

Major Findings: A. - Strains of Streptobacillus moniliformis have been isolated and characterized from atypical human infections and from cases of rat-bite fever. Acute and chronic lesions have been produced in mice and monkeys with these strains, and the organisms have been recovered on culture of the lesions.

9. (Cont'd)

Agglutination studies have been carried out in rabbits using human strain antigen. Several strains of this microorganism have been isolated from naturally-occurring lung and middle ear infections in old rats. Strains have been selected for ease of cultivation, and have been adapted to grow on relatively simplified culture media. Antigens have been prepared from the bacillary phase of the rat strains, and the phagocytosis of living and killed antigens by guinea pig blood leukocytes has been studied, with quantitation of levels of phagocytic activity.

Significance: A. - The development of a sensitive and simplified immunological method for detecting infection with, or immunity to, this microorganism will be of great value in cases where cultural recovery is difficult or impossible, and in confirming the significance of cultural recovery in infections. The occurrence of Streptobacillus moniliformis in atypical human infections may be responsible for clinical conditions without previously assigned etiology.

The relationship of Streptobacillus moniliformis to arthritis in rats may provide an experimental model for studying the possible infectious etiology of this disease in this and other species, and has direct bearing on a proposed cooperative project on experimental arthritis production with Dr. Leon Sokoloff, LPH-NIAMD.

B. - Despite the widespread belief that infection with the Streptococcus and resultant hypersensitivity are in some fashion involved in the pathogenesis of rheumatic fever, extensive cultural and serological studies for this microorganism in body fluids have failed to support this hypothesis. So far as can be ascertained, no attempt to date has been made to determine the presence or absence of specific sensitivity reactions in tissues, particularly in those tissues involved by this disease.

Proposed Course of Project: A. - Continue to screen human and animal infections culturally for Streptobacillus moniliformis. Supplement cultural diagnosis by immunological techniques for the detection of infection and for the characterization of strains.

Extend the measurements of phagocytosis of antigens prepared from rat strains to the determination of plasma opsonin levels in normal, immunized, and naturally and artificially infected animals. This will be correlated with cultural recovery and pathological examination.

9. (Cont'd)

Attempt to employ plasma opsonin determination or other immunological reactions as a means of detecting or confirming naturally-occurring infections with this microorganism in man and animals.

B. - Obtain tissues from cases of rheumatic fever, extract these, and by means of the currently available reactions to the Streptococcus, determine whether specific antibodies are concentrated in these tissues. Obtain tissues at various phases of the disease, from such sources as auricular appendage biopsies taken during mitral commissurotomy, subcutaneous nodules, etc. Prepare extracts by conventional techniques of grinding, macerating, etc., and eluting in quantitative fashion. Examine extracts for immunological reaction to the Streptococcus, employing reactions of varying degrees of specificity, such as: precipitin reaction, antistreptolysin-O, antistreptokinase, and antistreptodornase titres, etc. Determine tissue antibodies quantitatively and compare to blood levels of same, in order to establish whether if present and if concentrated in the lesions. Examine tissue specimens histologically concurrent with preparation of extracts.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-88
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

National Microbiological Institute; Laboratory of Infectious Diseases; Section on Bacterial and Mycotic Diseases and Section on Basic Studies. Provides facilities and personnel as needed.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN TH PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

This project complements and is part of research done by units described in 13, above, with interchange of personnel and facilities.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-88
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases — INSTITUTE
2. Pathology and Histochemistry — LABORATORY
3. Pathologic Anatomy — SECTION
4. _____ — LOCATION
5. NIAMD-89 — SERIAL NO.

6. Comparative pathology — PROJECT TITLE

7. Ladd N. Loomis — PRINCIPAL INVESTIGATOR(S)

8. A. W. Pratt and R. R. Williams — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Comparative pathology, both as individual and cooperative research.

(A). Rous Sarcoma of chickens (in cooperation with Dr. A. W. Pratt, NCI).

(1). Objectives: The histogenesis of the tumor was studied to determine the cell type involved in the formation of the Rous sarcoma.

Methods Employed: Purified Rous virus was inoculated subcutaneously into five week old chicks. Individual chicks were killed at intervals (ranging from 1 hour to 20 days) after inoculation and the inoculation site was examined microscopically for evidence of tumor formation. Routine histopathologic techniques and also special stains were used.

Findings: It was found that the tumor arose from the subcutaneous fibroblasts when the Rous virus was injected subcutaneously.

9. (Cont'd)

Significance: This is a contribution to our knowledge of tumors and their origins. The Rous sarcoma is in a special class and is the object of considerable controversy.

Proposed Course of Project: This phase is completed and a paper has been submitted for publication.

(2). Objectives: Using the material from (1) the skeletal muscle lesion caused by the Rous tumor is being studied to clarify its special characteristics.

Methods Employed: Microscopic examination of the earliest muscle lesions are being made. Special stains and some serial sections are being used.

Findings: The muscle lesion in the Rous tumor bearing chick arises in a characteristic manner considered to be different than the usual concepts of sarcomatous muscle lesions.

Significance: This study will add to our information on the Rous tumor with unknown significance for the cancer field.

Proposed Course of Project: A manuscript is being prepared.

(B). The development of a new technique for virus purification (in cooperation with Dr. R. R. Williams, NIAMD).

Objectives: To determine if the use of cellulose filtration combined with an antigen antibody complex will result in virus purification.

Methods Employed: Newcastle Disease virus (N.D.V.) was harvested from chick embryos and was ultracentrifuged. The ultracentrifuged virus material is exposed to powdered cellulose coated with anti-allantoic fluid rabbit anti-sera. A "before and after" calculation can be made, using LD_{50} ratios to nitrogen values in conjunction with hemagglutination titres, that identifies virus purification.

Findings: Some evidence of significant N.D.V. virus purification has been determined.

9. (Cont'd)

Proposed Course of Project: The work is continuing.

(C). Monkey safety tests of the Salk polio vaccine continues, in cooperation with Dr. L. L. Ashburn, NIAMD, for D.B.S.

Objectives: Examination of brain and spinal cord tissue sections from inoculated Rhesus and Cynomologous monkeys for evidence of live virus in the Salk polio vaccine.

(D). Neuropathology of dogs.

Objectives: Classification of the various CNS lesions of dogs is being made. Many of these lesions are rare and there is little information about them.

Methods Employed: A careful post-mortem examination is made and when combined with the history and the ante-mortem examination increases in value.

A systematic microscopic examination of the brain and spinal cord is made using standard histopathologic techniques and some special stains. Routine sampling of the other organs are examined microscopically.

Findings: The conditions encountered vary from virus diseases to traumatic conditions and tumors.

Proposed Course of Project: The survey is continuing and the interesting material will be published.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-89
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

9. (A). (1) and (2). Laboratory of Physiology, Radiation Physiology Section, National Cancer Institute. Dr. A. W. Pratt.

9. (B). Laboratory of Physical Biology, NIAMD. Dr. R. R. Williams.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

No parallel research in the Public Health Service.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-89
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases — INSTITUTE
2. Pathology and Histochemistry — LABORATORY
3. Pathologic Anatomy — SECTION
4. _____ — LOCATION
5. NIAMD-90 — SERIAL NO.

6. (A). Pathologic lesions induced by spermine and chemically related substances.
(B). Human pathology; diagnostic - research.
PROJECT TITLE

7. L. L. Ashburn
PRINCIPAL INVESTIGATOR(S)

8. Celia Tabor and Sanford Rosenthal
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

(A). Spermine Studies

Objectives: Study of the acute and chronic lesions induced by spermine and related substances in order to clarify mechanism of action.

Methods Employed: Administration of test substance and inactive (control) material by various routes (subcutaneous, intra-aortic and intra-arterial (renal)) and serial study of organs by gross and histologic methods.

Major Findings: Repeated subcutaneous administration of spermine results in moderate renal atrophy (guinea pig, rabbit), single intra-arterial (renal) administration causes an acute lesion which progresses to an irregularly diffuse renal atrophy and scarring, commonly associated with elevation of blood pressure (rabbit); focal myocardial necrosis.

Significance: The actual role of the kidney in renal hypertension is not known; another method of producing renal hypertension may be of help.

9. (Cont'd)

Proposed Course of Project: Continuation of attempts to determine mechanism of renal injury, correlation of renal alteration with blood pressure curve, methods of protecting kidney from injury by spermine, search for cause of cardiac hypertrophy and determination of relationship of myocardial necrosis to renal lesion.

(B). Human Pathology.

Diagnostic: Service to various Federal Hospitals, mainly those of the Division of Indian Health.

Research:

(1). Study of relative frequency of various tumor types in the American Indian by analyzing data in our files covering a period of about 20 years. The study at present is in the indexing phase.

(2). Study of the pathology of untreated syphilis in cooperation with venereal disease research program.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-90
SERIAL NO.

12. BUDGET ACTIVITY: Research.

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10).

Laboratory of Pharmacology and Toxicology, NIAMD (9. A).

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO(S). IF WITHIN NIH).

Complements spermine study done in LPT, NIAMD.

Analysis of NIH Program Activities

Honors, Awards, and Publication Sheet

15. NIAMD-90
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis & Metabolic Diseases
INSTITUTE

2. Pharmacology and Toxicology
LABORATORY

3. Biochemical Pharmacology
SECTION

4.
LOCATION (IF OTHER THAN BETHESDA)

5. NIAMD-91
SERIAL NO.

6. Biosynthesis of Nicotinic Acid
PROJECT TITLE

7. Alan H. Mehler
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To isolate the individual steps in the sequence of reactions resulting in nicotinic acid formation, to study the properties of the enzymes involved, and to describe the intermediate metabolites. With the reactions available, to study the relation of these enzymes to altered metabolic conditions.

Methods Employed: Enzymes are obtained from various sources and purified by the variety of methods currently used in this field. Chemical and physical, especially spectrophotometric, methods are used to measure enzyme activity and to identify products. Possible substrates and products are synthesized by conventional organic chemical techniques. Animals are treated to produce altered metabolic states, and enzymes from such animals are assayed.

Major Findings: During the last year it was found that a product of enzymatic action on 3-hydroxyanthranilic acid is picolinic acid, and that this compound is formed naturally and excreted as a glycine conjugate. In collaboration with Mr. E. McDaniel and Dr. J. Hundley, the enzyme that makes picolinic acid was found to be greatly increased in the livers of diabetic rats.

Significance to NIAMD Research: Two lines of inquiry are related to NIAMD research. One is a study of the reactions that influence niacin metabolism in order to gain more insight into the biochemistry of this vitamin. The other is the analysis of the effect of diabetes on liver enzymes, which may give information about the nature of the metabolic lesions in this disease.

Proposed course of project. During the next year it is proposed (1) to study factors influencing the level of liver enzymes as related to diabetes, (with Dr. Hundley and Mr. McDaniel) (2) to prepare isotopically labeled 3-hydroxyanthranilic acid (in collaboration with Dr. E. May) and use this material in studies on the metabolism of this compound in intact animals, in attempts to find the enzymes that synthesize niacin, and in a study of the nature of the picolinic acid-synthesizing enzymes, (3) to purify further enzymes involved in the metabolism of tryptophan and study the nature of the reactions catalyzed by them.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-91
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. None
IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 OR 1956; IF CO-OPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

14. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTER-CHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIAMD-91
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1954:

(1) Horecker, B. L. and Mehler, A. H. Carbohydrate Metabolism. Annual Review of Biochemistry. Vol. 24 (1955)

(2) Mehler, A. H. Formation of Picolinic and Quinolinic Acids Following Enzymatic Oxidation of 3-Hydroxyanthranilic Acid. J. Biol. Chem. In Press.

(3) Mehler, A. H. and Y. T. Chang Biological Reactions of Fluoroacetylcholine. Arch. Biochem. Biophys. In Press.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis & Metabolic Diseases
INSTITUTE

2. Pharmacology and Toxicology
LABORATORY

3. Biochemical Pharmacology
SECTION

4.
LOCATION (IF OTHER THAN BETHESDA)

5. NIAMD-92
SERIAL NO.

6. Histidine, Histamine & Related Imidazoles & Amines
PROJECT TITLE

7. Herbert Tabor
PRINCIPAL INVESTIGATOR

8. B. Ames, H. Bauer, E. Adams, O. H. Hayaishi (from Sect. on Toxicology)
PRINCIPAL INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To study the biosynthesis, intermediary metabolism, and pharmacological activity of these compounds in order to understand better their physiological and pathological role.

Major Findings: Further work has been done on the nature of the intermediate steps in the metabolism of histidine and histamine. A new synthesis of formiminoglutamic and of formiminoaspartic acid has been developed; these compounds are now available in large amounts. The former compound is an intermediate in the degradation of histidine, while the latter is now shown to be an intermediate

in the pathway histamine ⟶ imidazoleacetic and ⟶ formimino aspartic and ⟶ formylaspartic acid (in Pseudomonas with Dr. O. H. Hayaishi).

Studies on histidine biosynthesis have been carried out by Dr. Ames (1,2) and Dr. Adams (3,4).

(1) Imidazoleglycerol phosphate ⟶ imidazoleacetol phosphate.

(2) Imidazoleacetol phosphate ⟶ histidinol phosphate.

(3) Histidinol ⟶ histidinal.

(4) Histidinal ⟶ histidine.

The characteristics of these enzymes have been studied; in particular, enzyme 2 is a transaminase type enzyme with a pyridoxal phosphate cofactor.

A convenient synthetic method has been developed for the preparation of C^{14} and N^{15}- putrescine dehydrochloride. (See project report of Dr. S. M. Rosenthal on spermine.)

Significance to NIAMD Research: Histidine is an essential amino acid, and enters into many important metabolic relationships. The C-2 of the imidazole ring is important in "one-carbon metabolism", and is closely related to studies on the role of folic acid in metabolism. Changes in histidine metabolism have been described upon alterations in the hormonal pattern, particularly in rheumatoid arthritis. Histamine is a powerful pharmacological agent which is normally present in tissues. It appears to be very important in gastric secretions, neurohormonal reaction to stress, certain types of neural transmission, and in allergy and anaphylaxis. Histamine appears to be very important in stimulating the adrenal-anterior pituitary system. It is anticipated that further studies on its metabolic interrelationship will aid in clarifying histamine's role in these phenomena.

Proposed Course of Project: I. Histamine Metabolism

A. Nature of other urinary metabolites after administration of C^{14} histamine.

B. Enzymatic and chemical syntheses of imidazoleacetic acid riboside.

C. Enzymatic degradation of imidazoleacetic acid.

D. Physiological and pharmacological studies as indicated during the development of the project.

II. Histidine Metabolism

A. Purification of various enzymes involved in conversion of histidine to glutamic acid. Particular emphasis will be directed towards one-carbon transfer studies.

B. Biological syntheses and degradation of carnosine, anserine, ergothioneine, and other related imidazoles. Particular emphasis on studies concerned with the biosynthesis of histidine.

10. NIAMD-92
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. None. Funds for Dr. Hugo Bauer supplied by grant from National Science Foundation since August 1955; previous support from Ciba Co.
IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) ITEM 10)

14. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

15. NIAMD-92
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

(1) Bauer, H., Adams, E. and Tabor H. L-Histidinol Dihydrochloride. Biochem. Prep. Vol. 4, p. 46 (1955)

(2) Mehler, A. H., Tabor, H. and Hayaishi, O. Urocanic Acid. Biochem. Prep. Vol. 4, p. 50 (1955)

(3) Bauer, H. and Tabor, H. Cyanomethylimidazole and Imidazoleacetic Acid Hydrochloride. Biochem. Prep. Vol. 5, In Press.

(4) Tabor, H. Histamine - The Fate of Histamine in the Body. Ciba Symposium. (1955) In Press.

(5) Tabor, H. and Hayaishi, O. The Excretion of Imidazoleacetic Acid Riboside Following the Administration of Imidazoleacetic Acid or Histamine to Rats. J.A.C.S. 77, 505 (1955)

(6) Tabor, H. Degradation of Histidine. Amino Acid Metabolism. (1955)

(7) Tabor, H. Acetylation of Amines with Pigeon Liver Enzyme. Methods in Enzymology. Vol. I (1955)

(8) Tabor, H. and Mehler, A. H. Histidase and Urocanase. Methods in Enzymology. Vol. II (1955)

(9) Tabor, H. Isolation and Determination of Histidine and Related Compounds. Methods in Enzymology. (1955) In Press.

(10) Tabor, H, Tabor, C. W., and Rosenthal, S. M. Amine Oxidases. Methods in Enzymology (1955). In Press.

(11) Adams, A. Synthesis and Properties of An α-Amino Aldehyde, Histidinal. J.B.C. Vol. 217, No. 1 (1955)

(12) Adams, A. L-Histidinal, A Biosynthetic Precursor of Histidine. J.B.C. Vol. 217, No. 1. (1955)

(13) Ames, B. N. The Biosynthesis of Histidine. Amino Acid Metabolism. (1955)

(14) Ames, B. N. and Mitchell, H. K. The Biosynthesis of Histidine; Imidazoleglycerol Phosphate, Imidazoleacetol Phosphate, and Histidinol Phosphate. J.B.C. 212, 687 (1955)

(15) Ames, B. N. and Horecker, B. L. The Biosynthesis of Histidine; Imidazoleacetol Phosphate Transaminase. J.B.C. (1955). In Press.

17. None

LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis & Metabolic Diseases
INSTITUTE

2. Pharmacology and Toxicology
LABORATORY

3. Biochemical Pharmacology
SECTION

4.
LOCATION (IF OTHER THAN BETHESDA)

5. NIAMD-93
SERIAL NO.

6. Enzymatic Reactions Involved in the Synthesis of Metabolic Derivatives of Aspartic Acid and Methyl Mercaptan
PROJECT TITLE

7. Simon Black
PRINCIPAL INVESTIGATOR(S)

8. Mrs. Phyllis F. Downey (Previously Mrs. N. G. Wright)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: A long term objective is the discovery of enzymatic mechanisms involved in the synthesis of constituents of living tissue, particularly of proteins.

Methods: Cell-free enzyme extracts of yeast, as well as of plant and animal tissues, are tested for their ability to convert aspartic acid or methyl mercaptan to new compounds. Chemical, chromatographic, radiochemical, and radioautographic methods are used. When new substances are found the mechanisms of their formation are elucidated by classical enzymalogical methods.

Major Findings: It has been found that aspartic acid is converted to homoserine in yeast through three enzymatic steps involving the newly discovered intermediates, β-aspartyl phosphate and aspartic-β-semialdehyde.

It has also been found that methyl mercaptan is enzymatically incorporated into the previously unknown methylthiol ester of 3-phosphoglyceric acid, and that the latter is enzymatically converted to glyceryl methylthiol ester, also newly discovered, which has been isolated in crystalline form. An additional enzyme has been discovered in yeast which catalyzes the phosphorylation of glyceric acid by ATP to yield 3-phosphoglyceric acid.

Significance to NIAMD Research: Knowledge of the intimate chemical transformations in living cells will serve as a basis for better understanding of the nature of disease and its more intelligent treatment.

Proposed Course of Project: The immediate plan is to learn the relation of the newly found derivatives of methylmercaptan to established biochemical processes.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-93
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. None
IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 OR 1956: IF CO-OPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

14. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIA MD-93
SERIAL NO.

16. LIST OF PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Black, S. and Wright, N. G. β-Aspartokinase and β-Aspartyl Phosphate. Jour. Biol. Chem. 213 27 (1955)

Black, S. and Wright, N. G. Aspartic β-Semialdehyde Dehydrogenase and Aspartic β-Semialdehyde. Jour. Biol. Chem. 213 39 (1955)

Black, S. and Wright, N. G. Homoserine Dehydrogenase. Jour. Biol. Chem. 213 51 (1955)

Black, S. and Wright, N. G. Intermediate Steps in the Biosynthesis of Threonine, in "A Symposium on Amino Acid Metabolism" (Johns Hopkins Press) page 591 (1955)

Black, S. Potassium-Activated Yeast Aldehyde Dehydrogenase, in "Methods in Enzymology" (Academic Press) Vol. 1, pg. 508 (1955)

Black, S. and Wright, N. G. Enzymatic Formation of Glyceryl and Phosphoglyceryl Methylthiol Esters. Jour. Biol. Chem. In Press.

Black, S. A Microanalytical Method for Acetic and Other Volatile Acids, in "Methods in Enzymology" (Academic Press) Vol. 2. In Press.

17. None
LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Pharmacology and Toxicology
LABORATORY

3. Pharmacology
SECTION

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. NIAMD-94
SERIAL NO.

6. The physiological function, intermediary metabolism, and pathological effects of spermine, spermidine, and related amines.
PROJECT TITLE

7. Drs. S. M. Rosenthal, Celia W. Tabor, and R. C. Greene
PRINCIPAL INVESTIGATORS

8. Drs. H. Tabor, E. L. Jackson, and L. L. Ashburn (from LPH)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION.

Objectives: Spermine and spermidine have been found to be potent nephrotoxic agents. Analyses of animal tissues, and of plant and bacterial cells, have demonstrated a wide distribution, frequently in high concentration. Studies are conducted to elucidate its metabolism, physiological function, its relation to renal disease, to spermatozoal physiology, and to bacterial defense mechanisms.

Methods Employed: (1) The physiological effects of the degradation products (with an enzyme purified from beef plasma) have been further studied.

(2) Simplified chromatographic methods have made possible the rapid estimation of these amines in tissue extracts, body fluids, and in plants and bacteria.

(3) Administration to animals by various routes (including injection into the renal artery), to produce renal changes and hypertension.

(4) With isotopic labeled (C^{14} and N^{15}) putrescine, spermidine and spermine, the intermediary metabolism of these compounds can be followed.

(5) Mutant organisms requiring these amines for growth are being sought. An Aspergillus mutant (Sneath) is being studied.

Major Findings: (1) Spermidine has received little previous study due to lack of methods. A wide distribution has been found in animal and plant cells, in concentrations up to 1 mgm per gm. Evidence indicates that putrescine is also a normal constituent of animal tissues.

(2) These amines are not dietary in origin, being present in animals on purified diets, in the newborn, and in "sterile" rat liver (Notre Dame).

(3) A single injection of spermine or spermidine into the renal artery of rabbits causes progressive degeneration of the injected kidney, leading to almost complete atrophy in a few months. This is accompanied by hypertension and cardiac enlargement. Chronic subcutaneous administration produces atrophic changes in the epithelium of the cortex (with Dr. L. B. Ashburn).

(4) (With Dr. H. Tabor) The biosynthesis of spermine and spermidine from putrescine was shown in E. coli, yeast, Aspergillus, and minced prostate tissue by incubating with C^{14} and N^{15} putrescine. Putrescine is incorporated as a unit, since the C* - N* ratio is unchanged. Injection of labeled putrescine into rats resulted in labeled spermidine and spermine in the tissues.

(5) Spermine in the presence of the enzyme (amine oxidase) is highly lethal to spermatozoa and some bacteria (as low as 5 micrograms per ml.). This is antagonized by some SH compounds.

(6) A new method for the synthesis of spermidine was developed (Dr. Jackson).

NIAMD-94
SERIAL NO.

Significance to NIAMD Research: Spermine and spermidine are widely distributed in mammalian and plant tissues and no previous work has demonstrated any physiological function or relation to diseases. It is believed that this work will throw light on their biochemical significance, and that a causative relationship may be shown to some types of human renal disease.

Proposed Course of Project: (1) Study the characteristics of chronic lesions produced by spermine and spermidine, and their relation to human diseases.

(2) To establish the relationship of the pathological effects to the acute renal lesions which result from shock and eclampsia.

(3) To establish the significance of the spermatocidal and bacteriocidal actions in terms of spermatozoal physiology and of bacterial defense mechanisms.

(4) To further elucidate the intermediary metabolism of these amines by the use of mutant bacteria and isotopic laboling.

(5) Pharmacological effects, including renal function studies, following spermine administration.

10. NIAMD-94
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. Laboratory of Pathology & Histochemistry (Dr. L.L. Ashburn)
IDENTIFY ANY COOPERATING UNITS OF THE PHS, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 OR 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10).

14. This project does not resemble, complement, or parallel research done elsewhere in the PHS.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO(S) IF WITHIN NIH.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Rosenthal, S. M. and Tabor, Celia W. "The pharmacology of spermine and spermidine. Distribution and excretion." J. Pharm. & Exp. Therap., 1956;16.

Tabor, Celia W. and Rosenthal, S. M. "The pharmacology of spermine and spermidine. Some effects on animals and bacteria." J. Pharm. & Exp. Therap., 1956;16.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Laboratory of Pharmacology and Toxicology
LABORATORY

3. Pharmacology
SECTION

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. NIAMD-95
SERIAL NO.

6. Metabolic processes, drug action and physical chemistry related to electrolyte distribution and bioeleotrical phenomena as exemplified by nerve.
PROJECT TITLE

7. Dr. Abraham M. Shanes
PRINCIPAL INVESTIGATOR

8. Mr. Morris Berman
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION:

Objectives: To determine the effect of metabolic inhibition and substrates, of drugs with no demonstrable metabolic action but with a demonstrated effect on electrolyte distribution, and of alterations in the ionic milieu on the kinetics of entry and exit of sodium, chloride and potassium in nerve. The relation of these to the maintenance of cellular membrane potentials and to the production of action potentials also is to be established.

Method Employed: Desheathed sciatic nerves of the toad which our studies have demonstrated to be particularly favorable preparations, are exposed in vitro to radioisotope solutions and the kinetics of entry and exit are followed under conditions of metabolic inhibition and in the presence of substrates, drugs or modified medium. The variations of bioelectrical potentials under similar conditions are being followed with suitable electrical equipment.

NIAMD-95
SERIAL NO.

Major Findings: Techniques of study have been employed which permit a discrimination of ions located in the extracellular spaces from those in the axons proper. They also permit the study of the rates of entry and exit of the ions through the fiber membranes under strictly steady state conditions as well as under a variety of experimental conditions. Thus, it has been possible to show that the escape of sodium is not markedly affected by oxygen lack even when combined with inhibition of glycolysis and with treatment with dinitrophenol and eserine. However, return to oxygen following anoxia causes an acceleration of sodium efflux. The entry of potassium, on the other hand, is very sensitive to metabolic inhibition; it decreases to 1/3 or 1/4 when oxygen lack is supplemented by iodoacetate poisoning. Potassium exit increases about 50% under these conditions. The net movements of potassium, while usually balanced by a corresponding transfer of sodium, can occur without the involvement of sodium. A typical local anaesthetic, cocaine, has been found to have no effect on the metabolic transport of potassium, but depresses potassium transfer which occurs independent of metabolism.

Electrical studies presently under way permit examination of resting and action potentials under conditions that minimize the shifts in interstitial potassium concentration which occur under the moist chamber conditions usually employed. A depolarization occurs during anoxia which is appreciable in desheathed toad nerves but small in desheathed bullfrog nerves. The possible relation of this difference between the nerves from different species to diffusion limitations and to other factors is currently under investigation.

Single nerve fibers such as from the squid exhibit a phase which appears to be comparable to the extracellular space. In these preparations oxygen lack slowed sodium escape but accelerated potassium emergence. Cocaine slowed potassium efflux but not that of sodium.

NIAMD-95
SERIAL NO.

Significance to NIAMD Research: By the demonstration of the relation of metabolic processes and their derangement to normal functioning and malfunctioning of cells, as exemplified by nerve (which in many respects is quite similar to other cells of the body), a basis is being laid for an understanding of the nature of disturbances associated with metabolic diseases and for the development of means of treatment. The electrolyte studies to date emphasize that metabolic activity in nerve is geared in large measure to the selective inward transport of potassium rather than to the outward transfer of sodium. This may be true in muscle as well, since in this tissue interference with metabolism also leaves the outward movement of sodium unaffected. Attention is directed by these studies to the possibility that metabolic disturbances may lead to secondary effects resulting from potassium leakage. Moreover, our work suggests that the permeability of the system to cations, while partly dependent on metabolism, may also be altered directly without modifying the metabolic reactions themselves. This has two important implications: (1) The action of some drugs and hormones may be by way of their effects directly on the permeability of the cells to important physiological substances rather than, or in addition to, an effect on metabolism; this is now demonstrated for cocaine, appears to be the case for insulin, and has been suggested for cortisone; (2) derangements associated with the disturbance of electrolyte distribution may be delayed or possibly brought under control by the use of substances which act directly on the permeability of the cells.

Proposed Course of Project: (1) The movement of sodium, chloride, and potassium isotopes will be followed under similar experimental conditions; metabolic studies will be supplemented by an examination of the effects of alterations in the ionic milieu. (2) A correlation will be attempted between bioelectrical phenomena and the chemical findings; the possibility of extending our knowledge of anaesthetic action to the detailed ionic currents of activity demonstrable in giant squid fibers, will be explored. (3) An investigation will be begun on the possible role of carbohydrate metabolism in electrolyte distribution and transport, particularly as affected by related hormones.

10. NIA MD-95
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. None
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16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Shanes, A. M. (Editor) "Electrolytes in biological systems." Monograph published by the American Physiological Society, Washington, 1955.

Shanes, A. M. "Factors governing ion transfer in nerve." ELECTROLYTES IN BIOLOGICAL SYSTEMS (American Physiological Society), p.157, 1955.

Shanes, A. M. and Berman, M. D., "Penetration of the desheathed toad sciatic nerve by ions and molecules. I. Steady state and equilibrium distributions." J. Cell. Comp. Physiol. 45:177, 1955.

Shanes, A. M. and Berman, M. D. "Penetration of the desheathed toad sciatic nerve by ion and molecules. II. Kinetics." J. Cell. Comp. Physiol. 45:199, 1955.

Shanes, A. M. and Berman, M. D. "Kinetics of ion movement in the squid giant axon." J. Gen. Physiol. 39:279, 1955.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Dr. Shanes was asked to edit the Monograph "Electrolytes in Biological Systems," published in 1955 under the joint auspices of the American Physiological Society and the Society of General Physiologists.

NIAMD-95
SERIAL NO.

Dr. Shanes served as Chairman of the representatives of the Society of General Physiologists on the joint Local Arrangements Committee with the American Physiological Society for the Fall meetings held in Boston and Woods Hole, Mass., September, 1955.

Dr. Shanes was elected Secretary of the Society of General Physiologists for the period 1955-1957.

Dr. Shanes was an invited guest speaker at the international "Symposium on metabolic aspects of transport across cell membranes" held at the University of Wisconsin, August, 1955.

Dr. Shanes was elected to the Washington Academy of Sciences.

Dr. Shanes has been invited

(a) as a guest speaker on one of the panels in the conference on "The Electrophysiology of the Heart" being held at the N. Y. Academy of Sciences.

(b) to speak at a seminar at Princeton University

(c) to speak at a Biophysics Colloquim at Johns Hopkins University.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Pharmacology and Toxicology
LABORATORY

3. Pharmacology
SECTION

4. BETHESDA and LIMA, PERU, S. A.
LOCATION (IF OTHER THAN BETHESDA)

5. NIAMD-96
SERIAL NO.

6. Mechanisms and therapy of traumatic shock.
PROJECT TITLE

7. Drs. R. Carl Millican, Kohl Markley, and S. M. Rosenthal
PRINCIPAL INVESTIGATORS

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: (1) Study of the role of fluids, electrolytes and plasma proteins in cause and treatment of shock.

(2) Cause and therapy of late deaths in burns.

(3) Study of effectiveness and mechanisms of action of certain drugs against shock.

(4) Clinical evaluation in burn shock of the effectiveness of large volumes of saline solutions.

Methods Employed: (1) Standardized traumatic shock produced in mice by application of tourniquets to hind legs or dipping animals in hot water. Mortality studies, analyses of fluid and protein distribution and measurements of bleeding volume, hematocrit and plasma protein levels employed to study mechanisms for effectiveness of drug therapy in treatment of shock.

(2) The clinical study is divided into two parts. On an alternate case basis, (a) large volumes of saline solutions chiefly by mouth are compared with plasma therapy plus glucose solutions (b) large volumes of saline are compared with similar therapy supplemented by adequate plasma . The clinical study includes

mortality, fluid and electrolyte balances, hematological and hemodynamic measurements, and rate of gastrointestinal absorption in shock.

Major Findings: Experimental Shock. (1) Plasma administered during the period of swelling largely accumulates in the area of injury. When administered later, this does not occur, and under these conditions it is therapeutically more effective than when given early.

(2) Chlorpromazine and a metabolic product (Sulfoxide), given prophylactically, have a therapeutic effect in tourniquet shock. No reproducible benefit was observed from a variety of other drugs.

(3) Chlorpromazine increases blood volume and lowers plasma proteins and hematocrit, indicating movement of extravascular fluid into the circulation. These results indicate a possible therapeutic value preoperatively, in major surgery.

Clinical Study (Lima Project, Dr. Markley). Part 1 of the project with 100 cases each in the saline and plasma groups has been completed and submitted for publication. Mortality curves were similar in each group, demonstrating the effectiveness of oral saline as an emergency treatment of shock. No important difficulties in administration of saline were encountered and no serious hazards were observed.

In the problem of late deaths from burns, Pseudomonas septicemia with unusual skin lesions was found to be the major cause. A report of this work is being prepared for publication.

Significance to NIAMD Research: The mechanisms and therapy of shock are of general public health interest, and the use of oral saline as an emergency treatment is of major importance in Civil Defense and in Military Medicine.

NIAMD-96
SERIAL NO.

Proposed Course of Project: (1) Continued study of the mechanism of action and effectiveness of drugs in traumatic shock.

(2) Further experimental and clinical work on the causes and treatment of delayed death following extensive burns.

(3) Continuation of the clinical evaluation of saline and plasma therapy in burn shock.

10. NIAMD-96
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. Research Grant - NIH (Dr. K. Markley - Lima Project)
IDENTIFY ANY COOPERATING UNITS OF THE PHS, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 OR 1956: IF COOPERATING UNIT IS IN NIH INDICATE SERIAL NO(S).

14. This does not resemble, complement, or parallel research done elsewhere in the PHS.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO(S) IF WITHIN NIH.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Millican, R. Carl. "Traumatic shock in mice. Effectiveness of intraperitoneally administered whole blood, plasma, serum albumin in saline and saline." Am. J. Physiol. 181: 487-492, 1955.

Millican, R. Carl. "Tourniquet shock in mice. Comparison of serum and saline therapy administered early and late after injury." Am. J. Physiol. 183:187-192, 1955.

Millican, R. Carl and Stohlman, E. F. "Relative effectiveness of certain drugs against shock produced in mice from tourniquet and burn trauma." Am. J. Physiol. In press.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Pharmacology and Toxicology
LABORATORY

3. Pharmacology
SECTION

4.
LOCATION (IF OTHER THAN BETHESDA)

5. NIAMD-97
SERIAL NO.

6. The chemotherapy of mouse leprosy
PROJECT TITLE

7. Dr. Y. T. Chang
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The toxicology and evaluation of therapeutic effectiveness of drugs in leprosy. The tissue culture of intracellular parasites.

Major Findings: The quantitative evaluation of drugs in leprosy, using intraperitoneally infected mice, has given information closer to human mycobacterial infections than that given by mouse tuberculosis. Mice continuously treated for 15 months with a derivative of benzaldehyde of isonicotinyl hydrazone (Siebenmann's compound 377) revealed definite suppressive activity; while a similar derivative (Siebenmann's compound 373) and the parent compound, isoniazid, turned to be ineffective in prolonged treatment. Cycloserine was also effective in mouse leprosy. The evolution of mouse leprosy was studied in the intraperitoneally infected mouse, by the examination of the infected cells of the peritoneal smear and of the omentum. Using this technique, screening of drugs seems possible in animals treated for only 3 weeks. A simplified technique for tissue culture of monocytes infected with BCG seems possible.

NIAMD-97
SERIAL NO.

Significance to NIAMD Research: The leprosy studies are carried out in cooperation with the American Leprosy Foundation (Dr. Chang is on a fellowship from them). The results are applied to their clinical evaluation studies.

Proposed Course of Project: Continuation of evaluation of drugs in mouse leprosy. Continuation in the development of a short-term test in mouse leprosy. Continuation in the study of tissue culture of intracellular parasites.

10. NIAMD-97
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. Dr. Chang is supported by a Fellowship from the American Leprosy Foundation.
IDENTIFY ANY COOPERATING UNITS OF THE PHS, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 OR 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10).

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16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Chang, Y. T. "Chemotherapy of murine leprosy. III. The effects of nicotinamide and pyrazinamide (Aldinamide) on mouse leprosy." Internat. J. Leprosy, 1954; 22:331-346.

Chang, Y. T. "Chemotherapy of murine leprosy. IV. The effects of amithiozone (TB1/698), p-aminosalicylic acid (PAS), B283 (a phenazine pigment), five antibiotics and three diphenylthiourea compounds on mouse leprosy." Internat. J. Leprosy, '55;23:167-180.

Mohler, Alan H. and Chang, Y. T. "Biological reactions of fluoroacetylcholine." Arch. Biochem. & Biophys., in press.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Pharmacology and Toxicology
LABORATORY

3. Pharmacology
SECTION

4.
LOCATION (IF OTHER THAN BETHESDA)

5. NIAMD-98
SERIAL NO.

6. The synthesis of thyroxine analogs and related compounds.
PROJECT TITLE

7. E. L. Jackson
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The relation of new isomers of thyroxine and related compounds to physiological activity.

Major Findings: The synthesis of a meta isomer of thyroxine from known 2,6-dinitro-3-hydroxybenzaldehyde was started. 2,6-Dinitro-3-(p-methoxyphenoxy)-benzaldehyde was synthesized from 2,6-dinitro-3-(p-toluenesulfonyloxy)-benzaldehyde, which was prepared from 2,6-dinitro-3-hydroxybenzaldehyde. The crystalline pyridinium salt of 2,6-dinitro-3-(p-toluenesulfonyloxy)-benzaldehyde was isolated. This pyridinium salt is an intermediate in the formation of 2,6-dinitro-3-(p-methoxyphenoxy)-benzaldehyde by reaction of p-methoxyphenol with 2,6-dinitro-3-(p-toluenesulfonyloxy)-benzaldehyde in pyridine solution. 2,6-Dinitro-3-(p-toluenesulfonyloxy)-benzyl alcohol was obtained by reduction of 2,6-dinitro-3-(p-toluenesulfonyloxy)-benzaldehyde with aluminum isopropoxide. With 2,6-dinitro-3-(p-methoxyphenoxy)-benzaldehyde available from this work it should be possible to form the amino acid side chain and prepare a meta isomer of thyroxine.

NIAMD-98
SERIAL NO.

The synthesis of β-(3-methoxy-2,6-dinitrophenyl)-D,L-alanine from known 2,6-dinitro-3-methoxybenzaldehyde was started in order to prove the structure of one of the β-(3-hydroxydinitrophenyl)-D,L-alanines obtained in the course of this work by nitration of m-tyrosine. 2,6-Dinitro-3-methoxybenzyl alcohol was prepared by reduction of 2,6-dinitro-3-methoxybenzaldehyde with aluminum isopropoxide. 2,6-Dinitro-3-methoxybenzyl chloride resulted from the reaction of phosphorus pentachloride with 2,6-dinitro-3-methoxybenzyl alcohol. In order to form the alanine side chain, the reaction of ethyl acetamidomalonate with 2,6-dinitro-3-methoxybenzyl chloride is being studied.

With the exceptions indicated, all of the above compounds are new.

Significance to NIAMD Research: Some of the new compounds are possible thyroxine antagonists and also may be useful in studies on the relationship of chemical structure and thyroxine-like activity.

Proposed Course of Project: Continuation along the lines indicated under Major Findings.

10. NIAMD-98
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. None
IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 OR 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

14. This project does not resemble, complement, or parallel research done elsewhere in the PHS.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO(S) IF WITHIN NIH.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Jackson, E. L. "Diiodo-m-tyrosine, three isomeric dinitro-m-tyrosines and some of their derivatives." J. Am. Chem. Soc., 77:4860, September 20, 1955.

17. HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Laboratory of Pharmacology and Toxicology
LABORATORY

3. Toxicology
SECTION

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. NIAMD-99
SERIAL NO.

6. Metabolic studies on steroids and related compounds
PROJECT TITLE

7. Dr. O. Hayaishi
PRINCIPAL INVESTIGATOR

8. Dr. W. B. Jakoby and Mr. E. F. Stohlman
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: To elucidate the mechanism of biosynthesis, intermediate metabolism and degradation of steroids and related compounds.

Methods Employed: Through the enrichment culture technique, bacterial strains which rapidly metabolize steroids and related compounds were obtained from soil and animal feces. Highly active preparations of "adaptive enzymes" were produced by these microorganisms when they were grown on specific substrates. By the use of such highly active enzyme preparations, the detailed metabolic pathways were investigated.

Major Findings: A new adaptive enzyme, 3-α-hydroxybile acid dehydrogenase was isolated from a bacterium and its properties were investigated. Similar enzyme was found in mammalian liver. These enzymes play an important role in the metabolism of bile acids and steroids.

For example, a possible detoxification mechanism for a steroid anesthetic, Viadril, (sodium pregnan-21-ol-3,20-dione-21-hemisuccinate) was studied with various enzymes from rat liver. Reduction of Viadril to 3-hydroxy derivative by the bile acid dehydrogenase appears to be a primary step in the detoxification mechanism.

Significance to NIAMD Research: Steroids have been found to be most important for the treatment of arthritis and certain metabolic diseases. Studies on the metabolism of this group of compounds will throw some light on the understanding of these diseases and may ultimately lead to better treatment.

Proposed Course of Project: Metabolism of steroids, bile acids and related compounds will be studied by enzymes from microbial and mammalian origin.

10. NIAMD-99
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. Dr. Y. Sato, NIAMD, LC, SS, has been col orating on this project.

Dr. C. Tomkins, CI, NIAMD, has been collaborating on Viadril metabolism.

IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

14. None

IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUELIC HEALTH SERVICE (WITHOUT INTEREXCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

15. NIAMD-99
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

"Reversible Enzymatic Oxidation of Bile Acids".
O. Hayaishi, Y. Sato, W. B. Jakoby and
E. F. Stohlman, Arch.Biochem.Biophys., 1955:
56, 554.

"An Enzymatic Detoxification Mechanism for Viadril".
W. B. Jakoby and G. Tomkins, Science, in press.

17. None
LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Laboratory of Pharmacology and Toxicology
LABORATORY

3. Toxicology
SECTION

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. NIAMD-100
SERIAL NO.

6. Toxicologic studies of iodates, the cellular localization of hyaluronidase.
PROJECT TITLE

7. Dr. S. H. Webster, Dr. W. F. von Oettingen, and Dr. E. W. Emmart
PRINCIPAL INVESTIGATOR(S)

8. Mr. E. F. Stohlman
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION:

Objectives: The ' icology of iodates as a basis for use in iodized salt to prevent goiter. By the use of fluorescein labeled antibody to streptococcal hyaluronidase to determine the cellular localization of this enzyme in mammalian tissues.

Method Employed: Administration of iodates and iodides to experimental animals and subsequent examination by hematological, ophthalmological and histopathological methods. Use of fluorescein labeled antibody and fluorescence microscopy.

Major Findings: Administered orally to mice or dogs, KIO_3 is rather rapidly converted into iodide and eliminated in the urine. After large doses of iodate, both iodate and iodide appear in the urine. Iodate

in the stomach appears to inhibit temporarily the secretion of hydrochloric acid in rodents and dogs.

A suitable method for the daily oral administration of KIO_3 has been worked out for dogs; the upper dosage limit of tolerance has been determined; the acute lethal dosage range has been estimated; and the action of this drug, with respect to weight change, appetite, blood and urine findings, and gross and histopathologic changes has been investigated. Methemoglobinemia was not found to be induced in dogs fed sublethal doses of KIO_3. The study of the use of organ-body-weight relationships has been continued. (Webster)

A survey of the clinical toxicity of antibiotics and halogenated hydrocarbons was made to evaluate their hazards in clinical and industrial use. (von Oettingen)

An improved method of cultivation of streptococcus group C strain 7 and partial purification of hyaluronidase from this organism were described. The enzyme is antigenic in rabbits. The localization of injected hyaluronidase in the eyes of experimental animals and that of chorionic gonadotropin in rats were studied by the use of fluorescent antibody. (Emmart)

Significance to NIAMD Research: The use of iodate to iodinize salt is a problem of importance in tropical countries, where volatile and hygroscopic properties of KI present a difficulty. Studies with hyaluronidase may throw some light on the aetiology and treatment of arthritis and rheumatic diseases.

Proposed Course of Project: A chronic toxicity feeding experiment, using three levels of KIO_3, is being started with dogs. This will last for one year. Electroretinograms and photographs of the fundi will be made in cooperation with Drs. Gunkel and Bornschein of the NINDB, at the beginning and end of the experimental period. (Webster)

Survey of the toxicologic evaluation of drugs will be continued. (von Oettingen)

Histochemical studies on the localization of injected hyaluronidase and of human gonadotrophic hormone will be continued. (Emmart)

10. NIAMD-100
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. Dr. Ben Highman, NIAMD (Pathology) and Dr. Ralph Gunkel, NINDB (Retinal Examinations) (with Dr. Webster).

Dr. R. N. Cole, NMI, J. P. Longley, NIAMD, L. V. Sallman, INDB, A. Breslow, DBS, and H. Altman, NCI, (with Dr. Emmart).
IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956; IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

14. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTEREXCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

15. NIAMD-100
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

"Organs Body-weight Ratios for Certain Organs of Laboratory Animals. III. White Swiss Mouse". S. H. Webster and E. J. Liljegren, Am.J.Anat., 1955: 97, No.1, p.129.

"Complications of Antibiotic Therapy". W. F. von Oettigen, Am.J. of Med., 1955: 18, 792.

"The Halogenated Hydrocarbons, Their Toxicity and Potential Dangers". W. F. von Oettingen, Public Health Publication No. 414, U. S. Government Printing Office, 1955: p.429.

"Handbook of Toxicology, Vol. I". W. F. von Oettingen, published by the Committee on the Handbook of Biological Data, Division of Biology and Agriculture, The National Academy of Sciences, The National Research Council, 1955.

"Studies on Streptococcal Hyaluronidase and Antihyaluronidase. I. The Development in Vitro of Streptoccal (Group C) Hyaluronidase, Its Isolation and Use as an Antigen in Rabbits". E. W. Emmart and R. H. Cole, J. of Bact., 1955: 70, No.5, Nov., p.596-607.

17. None
LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Laboratory of Pharmacology and Toxicology
LABORATORY

3. Toxicology
SECTION

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. NIAMD-101
SERIAL NO.

6. Metabolism of dicarboxylic acids
PROJECT TITLE

7. Dr. O. Hayaishi
PRINCIPAL INVESTIGATOR

8. Dr. W. E. Jakoby, Dr. E. Ohmura and Dr. Y. Ogura
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: To elucidate the mechanism of biosynthesis and degradation of biologically important dicarboxylic acids.

Methods Employed: Through the enrichment culture technique, bacterial strains which rapidly metabolize dicarboxylic acids were obtained from soil. Highly active preparations of "adaptive enzymes" were produced by these microorganisms when they were grown on specific substrates. By the use of such highly active enzyme preparations, the detailed metabolic pathways were investigated. Chemical and spectrographic methods were employed together with O_2^{18} and H_2O^{18} to study the intimate mechanism of individual reactions.

Major Findings: Oxalic acid decarboxylase was isolated and purified from a bacterium. ATP, CoA, Mg ion, cocarboxylase and acetate were found to be required.

NIAMD-10E
SERIAL NO.

This finding extends our previous observation on malonate decarboxylase to another substrate and indicates rather ubiquitous distribution of this novel type of decarboxylation reaction in nature. Experiments with H_2O^{18} indicated that the mechanism of enzymatic decarboxylation of malonate was β-decarboxylation of monomalonyl CoA.

Significance to NIAMD Research: In spite of the recent progress in the knowledge of fatty acid metabolism, little has been understood about the metabolism of dicarboxylic acids.

Oxalic acid has been known for years to be widely distributed in men, plants and microorganisms and together with malonic acid, exhibits high toxicity in men. Knowledge concerning how these dicarboxylic acids are metabolized in living cells will serve as a basis for better understanding of certain metabolic diseases.

Proposed Course of Project: Further studies on the enzymes and metabolism of oxalate, malonate and adipate. A new type of oxalic decarboxylase from wood-destroying fungi will be studied with regard to its properties and the reaction mechanism.

10. NIAMD-101
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. Mr. S. Rothberg, NHI, LCPM, has been collaborating in O^{18} experiments.
IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

14. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTEREXCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

15. NIAMD-101
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

"Enzymatic Decarboxylation of Malonic Acid". O. Hayaishi, J.Biol.Chem., 1955: 215, 25.

"$C^{14}O_2$ Fixation in Microorganisms". O. Hayaishi, Kagaku-no-ryoiki, in press.

17. None
LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Laboratory of Pharmacology and Toxicology
LABORATORY

3. Toxicology
SECTION

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. NIAMD-102
SERIAL NO.

6. Metabolism of aromatic amino acids and related compounds
PROJECT TITLE

7. Dr. O. Hayaishi
PRINCIPAL INVESTIGATOR

8. Dr. M. Katagiri, Dr. E. Ohmura, and Dr. W. B. Jakoby
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: To study the metabolism and pharmacological activity of aromatic amino acids and related compounds.

Methods Employed: Through enrichment culture and adaptive enzyme technique, enzymes were isolated and purified from various microorganisms which catalyze chemical transformation of biologically important aromatic compounds. Chemical and spectrographic methods were used together with O_2^{18} and H_2O^{18} to study intimate mechanisms of individual reactions.

Major Findings: By the use of O^{18}, pyrocatechase, an enzyme which ruptures the benzene ring, was found to be a new type of oxygen-transferring enzyme. In addition to pyrocatechase, homogentisicase, 3-hydroxyanthranilic acid oxidase and protocatechuic acid oxidase appear to belong to this new class of

metallo-protein enzymes which introduce two oxygen atoms directly across the aromatic bond adjacent to the phenolic group with simultaneous rupture of the aromatic structure.

β-ketoadipic acid, a metabolite of a number of aromatic compounds, is now shown to be metabolized by a specific enzyme in the presence of succinyl CoA and CoA to produce a stoichiometric amount of succinate and acetyl CoA.

Kynurenine transaminase has been purified from Neurospora and its properties were studied.

Significance to NIAMD Research: Aromatic amino acids and related compounds are of great biochemical importance. It is hoped that elucidation of their metabolism will lead to better understanding of their nutritional, pharmacological and toxicological activities.

Proposed Course of Project: The work will be pursued to establish individual steps and obtain thorough understanding of their metabolic pathways. Studies with O_2^{18} and H_2O^{18} to investigate the mechanism of enzymatic hydroxylation and oxidation of various compounds will be continued.

10. NIAMD-102
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. Mr. S. Rothberg, NHI, LCPM, has been collaborating in O^{18} experiments.

Similar type of work on the metabolism of imidazole acetic acid has been carried out in collaboration with Dr. H. Tabor and details will be reported from the Section on Biochemical Pharmacology.
IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

14. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTEREXCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

15. NIAMD-102
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

"Enzymatic Studies on the Metabolic Interrelationship of Hydroxy-substituted Derivatives of Tryptophan". O. Hayaishi, Amino Acid Metabolism, 1955: p.914.

"Enzymatic Studies on the Metabolism of Imidazole Acetic Acid". O. Hayaishi, Amino Acid Metabolism, 1955: p.391.

"The Excretion of Imidazole Acetic Acid Riboside Following the Administration of Imidazole Acetic Acid or Histamine to Rats". H. Tabor and O. Hayaishi, J.Am.Chem.Soc., 1955: 77, 505.

"Mechanism of Pyrocatechase Reaction". O. Hayaishi, M. Katagiri and S. Rothberg, J.Am.Chem.Soc., 1955: 77, 5450.

"Application of Radioactive Isotopes to Biochemistry". O. Hayaishi, 1955: Seikagaku, 27, 1.

"Kynurenine Transaminase". W. B. Jakoby and D. Bonner, J.Biol.Chem., in press.

17. Dr. O. Hayaishi was invited by the Japanese Medical Association to address at the 14th General Assembly of the Medical Congress of Japan in April 1955. The address entitled "Application of Radioactive Isotopes in Biochemistry" was later published in Seikagaku (Journal of the Japanese Biochemical Society), Vol. 27, p.1, 1955.

LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

Analysis of NIH Program Activities

January-December 1955

National Institute of Arthritis and Metabolic Diseases
Clinical Investigations

Studies on Adrenal and Synthetic Steroids

Prednisone, a synthetic steroid chemically related to cortisone, was introduced into clinical medicine by National Institute of Arthritis and Metabolic Diseases scientists in the fall of 1954. The original findings that this new drug was four times more potent than cortisone or hydrocortisone, that it was a very effective antirheumatic agent, that it was free from troublesome effects of causing retention of sodium and water or loss of potassium, that it brought pronounced relief to arthritics who had stopped responding to cortisone, hydrocortisone, or ACTH -- all these findings have since been amply confirmed by hundreds of physicians in this country and abroad who had administered prednisone to thousands of patients. The advantages of prednisone are so evident that it is currently replacing cortisone and hydrocortisone.

During the past year, studies by the National Institute of Arthritis and Metabolic Diseases investigators have been extended to other rheumatic diseases besides rheumatoid arthritis. The clinical, hormonal, and metabolic effects of prednisone in groups of patients with acute rheumatic heart disease, systemic lupus erythematosus, and scleroderma have been recently completed and reported in scientific medical periodicals. In all these diseases the new drug was found to produce striking improvement without causing disturbances in the delicate mineral balance of the body.

Prednisone, however, is not free from serious, unwanted side effects, nor can it be regarded as a cure for any of these diseases. For these reasons the search for a more satisfactory drug -- one that would be relatively free of undesirable reactions and yet retain potent antirheumatic properties -- is being actively conducted.

A new synthetic compound, delta-one, 9 alpha, fluorohydrocortisone, has been subjected recently to clinical trial for the first time, at the National Institute of Arthritis and Metabolic Diseases. A group of ten patients

with rheumatoid arthritis was given this steroid. It was found that this drug was 5 to 10 times more potent than prednisone or 20 to 40 times more potent than cortisone, but unfortunately it caused marked retention of sodium and water and severe loss of vital potassium salts. For these reasons this drug will not be clinically useful in the treatment of rheumatoid arthritis and other rheumatic diseases. It may have an important place, however, in the management of certain disorders of the adrenal cortex, such as Addison's disease and the adreno-genital syndrome or in maintaining adrenalectomized patients with neoplastic diseases.

Concurrent with these clinical studies, more fundamental laboratory research work has been done at the National Institute of Arthritis and Metabolic Diseases. Employing more accurate methods than have been available previously, National Institute of Arthritis and Metabolic Diseases scientists have measured precisely the amount of hydrocortisone that the normal human adrenal cortex synthesizes daily, the rate at which this hormone disappears from the blood into the liver and out through the kidneys. It was found that stimulation by corticotropin resulted in a six-fold increase in endogenous secretion of hydrocortisone. Although the miscible pool of hydrocortisone in man was observed to be normal in diabetes, cirrhosis of the liver, myxedema and thyrotoxicosis, the rate of synthesis of this hormone was markedly reduced in cirrhosis of the liver and myxedema, accelerated in thyrotoxicosis and normal in diabetes.

The enzyme systems in the liver that are responsible for inactivation (reduction and conjugation) of hydrocortisone and similar hormones have been defined. This new knowledge will lead to a better understanding of how the body normally disposes of the steroids it synthesizes. It also will offer a sound basis for intelligent administration of this compound to patients with a wide variety of diseases. It may lead to specific enzymatic procedures by which the concentrations of various corticosteroids in the blood can be measured.

Distinctive Character of Serum Proteins in Rheumatoid Arthritis Defined

Advances have been made by Institute scientists in our knowledge of the nature of the protein components in the sera of patients with rheumatoid arthritis. It was

demonstrated during the past year that gamma globulin from normal sera inhibits the hemagglutination of sensitized sheep cells by sera from patients with rheumatoid arthritis. The fraction of normal gamma globulin responsible for the inhibitory activity was separated electrophoretically and found to constitute but a small portion of the total gamma globulin. It was demonstrated that inhibition was effected by binding of gamma globulin with the agglutinating factor of rheumatoid serum. The quantity of bound gamma globulin could be measured precisely. The rheumatoid agglutinating factor recovered in the euglobulin was fractionated by extraction at low salt concentration into agglutinating and inhibitory components, the latter predominantly gamma globulin. It was discovered that inhibition of the agglutinating factor could also be effected by certain organic compounds such as glycyl glycine, ureidosuccinic acid, 5-acetic hydantoin and glucosamine. It now seems likely that this work will achieve two important objectives. First, the interaction between certain protein fractions separated from rheumatoid and normal serum may prove to be specific, hence diagnostic for rheumatoid arthritis. This would eliminate two troublesome and extraneous biological elements from the current serological test for this disease; namely, the sheep erythrocyte and rabbit antiserum. Secondly, the quantity of certain protein fractions in rheumatoid serum bound or left unbound could now be measured with precision, thus subjecting the test, for the first time, to satisfactory quantitative analysis.

Intermediary Uric Acid Metabolism in Gout

Previous studies by Institute scientists of the incorporation of 4-amino-5-imidazole carboxamide-4-C^{13} (AIC-C^{13}) into uric acid in normal subjects have been extended this year to patients with gout. Two gouty patients who incorporated glycine-N^{15} into uric acid in a manner similar to normal subjects showed a pattern of incorporation of AIC-C^{13} into uric acid that also closely resembled normal subjects in that there were two pathways for this incorporation. There was some evidence to suggest an increased magnitude of incorporation in the gouty patients. As in the normal subjects, AIC depressed the de novo synthesis of uric acid from glycine.

Following oral administration of glycine-1-C^{14}, the isotope concentration in urinary purines (uric acid, xanthine, hypoxanthine, adenine) were determined and compared over 8-14 day periods in normal and gouty subjects. In both types of subjects, uric acid was more

highly enriched than any of the other three purines at all points in time, except that hypoxanthine enrichment exceeded that of uric acid on the first day. From the shapes of the various curves, it would appear that in both normal and gouty subjects there is evidence that uric acid is synthesized via hypoxanthine by a rapid pathway, and also via adenine by a slower pathway. In one gouty "overproducer" evidence suggested that the early pathway involving hypoxanthine was utilized to a greater extent than in the control subject. The findings with glycine-1-C^{14} to date are in agreement with the hypothesis that there are two major routes of urate synthesis in man, one a rapid shunt pathway, the other a slower, quantitatively more important pathway involving nucleic acids as intermediates. The data obtained with glycine-C^{14} suggest that the rapid shunt pathway of urate synthesis may involve hypoxanthine (free or combined with ribose ± phosphate) as an intermediate. They further raise the question whether urate may also be synthesized via pathways not involving free hypoxanthine and xanthine as intermediates.

Diabetes

It has been demonstrated by Institute scientists that when patients with diabetes develop acidosis there appears in the blood an activity which antagonizes that of insulin. This discovery explains in large part the increased insulin requirement of diabetic patients during episodes of acidosis. The anti-insulin activity is demonstrated by an *in vitro* technique and has been found to disappear promptly when treatment directed against acidosis is instituted. The material responsible for this activity is not one of adrenal cortical steroids. It is either a large molecule or largely associated with large molecules and behaves chemically as though it were in the globulin fraction of the plasma protein. Its chemical nature, its tissue of origin and the mechanism whereby it opposes the action of insulin are all subjects of current study.

During the past year, studies done on normal subjects revealed that glucose is not the only sugar that is influenced by insulin. Of those sugars thus far studied, two pentoses, namely, D-xylose and L-arabinose, intravenously administered, disappear from the blood at significantly increased rates when insulin is given. D-arabinose does not respond to insulin in a similar manner. It is now evident that in man, as well as in the dog, insulin affects the distribution in body fluids of D-xylose and L-arabinose but not D-arabinose.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Arthritis and Rheumatism
LABORATORY OR BRANCH

3. Clinical Investigations
SECTION

4. _____

5. NIAMD-103-c
SERIAL NUMBER

6. Trial of New Antirheumatic Drugs
PROJECT TITLE

7. Joseph J. Bunim, Roger L. Black, Alfred J. Bollet, Gerald P. Rodnan
PRINCIPAL INVESTIGATORS

8. G. Donald Whedon, Ralph E. Peterson, Stanton Segal, K. Lemone Yielding
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To evaluate the antirheumatic and hormonal potency and to determine the metabolic effects and therapeutic limitations of new synthetic steroids.

Methods Employed: Clinical appraisal and measured response of patients with rheumatic diseases to new compounds. Metabolic and hormonal studies of these drugs are also done.

Patient Material:

		Number	Average Stay in Days
Admissions:	Adult males	17	73
	Adult females	30	79
	Children males	--	--
	Children females	3	73
Outpatient:	No. of patients	42	
	No. of visits	63	

Major Findings:

Rheumatoid Arthritis

Prednisone and prednisolone were clinically evaluated in a group of approximately 30 patients with rheumatoid arthritis and detailed studies were done in 18 of these. These new steroids were found to be effective antirheumatic agents, 4 times more potent than cortisone or hydrocortisone. Pain, tenderness, and warmth disappeared within 24-48 hours of administration. Swelling decreased more slowly over two to five days. Synovial biopsies showed a marked

subsidence of acute synovial inflammation. Improvement in the clinical status continued on maintenance doses of 10 to 30 mg. daily. In several patients, it was necessary to increase the daily dose by 2.5 to 5.0 mg. to maintain the improved status.

In therapeutic doses, no sodium retention or potassium diuresis was observed. In the group of 18 patients, several serious side effects of steroid administration were observed. Four patients developed peptic ulcer, 4 had collapsed vertebrae, 3 developed mental disturbance, and 10 had evidence of interference with carbohydrate metabolism. Facial rounding, restlessness, and petechial hemorrhage were also common.

In spite of maintained clinical remission, evidence of progressive bone damage was observed in two patients.

Progressive Systemic Sclerosis

Six patients with progressive systemic sclerosis were given prednisone in daily doses of 30 mg., gradually tapered to 10 to 20 mg. Five of the patients have been followed 7 to 9 months and have continued to show symptomatic and objective improvement. The skin softened in most areas and the ulcers on finger tips healed. Involved joints consistently showed decrease of pain, swelling, and tenderness, although synovial biopsies failed to show decisive subsidence of inflammation during the treatment period. Dysphagia disappeared although the radiologic pattern of sclerodermal involvement of the esophagus persisted in two patients. Raynaud's phenomenon tended to persist.

Serious side effects have not been observed in the 5 patients followed over the 7 to 9 month period. The status of the 6th patient is not known.

Systemic Lupus Erythematosus

These two new steroids were also evaluated in 10 patients with systemic lupus erythematosus in doses of 20 to 60 mg. per day. Favorable response was observed in fever, chills, skin, and mucous membrane lesions, pleuritic pains and friction rubs, abdominal pain and mental changes in all patients showing these manifestations of LE activity. Arthralgia, dyspnea, nausea, vomiting, and headaches disappeared in most instances. Very little favorable effect was noted in the edema, hypertension, retinopathy, proteinuria, and hematuria in patients with renal damage.

The patients were followed for 2 to 8 months. Two died of renal failure. Minor side effects occurred in all 10. Facial rounding and hirsutism were common. Euphoria was noted in 2. No serious side effects, however, were encountered.

The metabolic, hormonal, and antirheumatic effects of delta-1, 9 alpha-fluorohydrocortisone were investigated in a group of 10 patients with rheumatoid arthritis and in two control patients, one normal and one with osteoarthritis. This new steroid was shown to cause marked sodium retention and increased potassium excretion. It was found to be 10 times more potent than prednisone in its capacity to inhibit corticotropin secretion by the adenohypophysis.

The antirheumatic potency was approximately 5 times that of prednisone in those patients who could tolerate a therapeutic dose of delta-1-FF. In most instances, the development of severe edema precluded raising the daily dose of delta-1-FF above 4 mg. Hypokalemia was a frequent undesirable side effect, occurring in 6 patients. One of 5 patients who had glucose tolerance tests before and during therapy, showed the development of a diabetic curve. Facial rounding was noted in 7 patients.

Significance to NIAMD Research: There is great need for a more satisfactory therapeutic agent in rheumatoid arthritis and other rheumatic diseases. It is hoped that further clinical trials at NIAMD may provide information which will assist in the synthesis of more effective and less toxic agents.

Proposed Course of Project: The search will be continued for new synthetic steroids which would retain anti-inflammatory potency and yet be free of potent mineral effects.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-103-c
SERIAL NUMBER

12. Research
BUDGET ACTIVITY

13. No cooperating units.
COOPERATING UNITS OF PHS

14. No parallel research in PHS.
PARALLEL RESEARCH IN PHS

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

16. PUBLICATIONS FROM THIS PROJECT

Studies on Metacortandralone and Metacortandracin in Rheumatoid Arthritis. J. J. Bunim, M. M. Pechet, and A. J. Bollet. J.A.M.A., 157:311, 1955.

Metabolic Effects of Metacortandralone and Metacortandracin. J. J. Bunim, R. L. Black, A. J. Bollet, and Pechet, M. M. Annals of the New York Academy of Sciences, 61:358, 1955.

Major Undesirable Side-effects Resulting from Prednisolone and Prednisone. A. J. Bollet, R. L. Black, and J. J. Bunim. J.A.M.A., 158:459, 1955.

Treatment of Systemic Lupus Erythematosus with Prednisone and Prednisolone. A. J. Bollet, S. Segal, and J. J. Bunim. J.A.M.A., 159: 1501, 1955.

17. No honors or awards.
HONORS AND AWARDS

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Arthritis and Rheumatism
LABORATORY OR BRANCH

3. Clinical Investigations
SECTION

4. _____

5. NIAMD-104-c
SERIAL NUMBER

6. Studies in Anemia
TITLE OF PROJECT

7. Franklin G. Ebaugh, Jr.
PRINCIPAL INVESTIGATOR

8. Ralph E. Peterson, Gerald P. Rodnan, Ted Clemens, Jr.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: A study of the mechanism and the rate of the elution of chromium-51 from circulating red cells in vivo.

The application of sodium chromate and Ashby selective agglutination techniques in the study of various anemias in the human, and the use of Cr51 for comparative red cell survival in various animals.

Methods Employed: Methods employed in this project have previously been described in projects for calendar year 1954. "Measurement of red blood survival in vivo by means of radioactive sodium chromate labeled red cells and Ashby Selective Agglutination and iron metabolism by radioactive Fe59 tracer techniques and the chemical determination of the serum iron employed together with concurrent clinical studies".

Patient Material: Anemic patients on the clinical NIAMD service (in-patient service) and out-patients, NIH employees who served as normal volunteers, normal volunteers who were in-patients, and one patient who was an out-patient at Howard Medical School and Freedman's Hospital who was studied in collaboration with Dr. William Boler, the hematologist at Freedman's.

		Number	Average Stay in Days
Admissions:	Adult males	16	59
	Adult females	16	78
	Children		
OutPatient:	Number of patients	27	
	Number of visits	1018	

Major Findings: The manner in which the sodium chromate was eluted in vivo from circulating erythrocytes was further studied by the combination of Ashby selective agglutination and sodium chromate tagging. Previous work indicated that from the normal erythrocyte, sodium chromate eluted at a rate with a half-time of 77 days. It was the purpose of the study of 1955 to see whether the rate was altered in red cells which were either abnormal intrinsically or circulating in an abnormal environment. Red cells obtained from normal subjects, patients with rheumatoid arthritis, sickle cell anemia, and a patient with hereditary spherocytosis were utilized. The conclusions from these studies indicated that in the normal individual the elution of chromium appeared to be initially more rapid and had a half life of 6 days during the first few days; thereafter, the rate of elution was found to be substantially what it had been found before--namely, with a half-time of 80 days. It was further shown that cell survival studies employing either the Ashby selective agglutination technique or Cr51 must receive special interpretation during the first two days since events which have little or nothing to do with the rate of red cell destruction occur. There is a drop of about 6% during the first 24 hours by either Ashby transfused cells or chromium-tagged cells. It is felt that this drop is not due to increased cell destruction, since the same phenomenon occurs with cells tagged in vivo, nor is this drop due to the increased rate of elution of chromium known to occur during this period. It is concluded that the initial drop is due to the fact that transfused red cells fail to equilibrate fully with all the red cell mass and that the process of equilibration which occurs 24-48 hours after transfusion accounts for this drop.

It was found that the rate of elution of sodium chromate-51 from abnormal red cells such as congenital hemolytic jaundice and those red cells obtained from patients with active rheumatoid arthritis was not significantly different from normal. The rate of chromium elution from sickle cells was slower than that seen in the normal, and indeed no elution of sodium chromate-51 from transfused sickle cells in vivo could be demonstrated.

Comparative study of red cell survival on a normal male and female: Previous studies utilizing the Ashby technique have indicated a slightly shorter survival of normal transfused blood in the female than in the male, but no data were published involving the survival of autotransfused cells in the female. Accordingly, 19 normal females and 10 normal males were studied and the results compared. It was found that although the survival of the normal females was slightly less than the males, this difference was very slight indeed and of no statistical significance. It is therefore concluded that the rate of survival of autotransfused chromium tagged red cells in the normal male and female is the same.

The comparative aspects of red cell survival was studied, employing sodium chromate tagged red cells in vivo in the chicken, duck, pigeon,

and rat. It was found that the maximum survival of the nucleated avian cells in the 3 species studied was between 30 and 40 days. The rat was found to have a maximal life span of 50 days. A comparison of the rate of red cell turnover in all the animal species in which this data is known and metabolic rate was made and a highly significant direct comparison was found between the rate of red cell turnover and the metabolic rate in the various species compared. As a part of this study, the red cell survival of the turtle erythrocyte was studied in 2 turtles. The results indicated that the turtle erythrocyte has a maximum survival of between 500-700 days. This finding fits very well with the low metabolic rate of the turtle. In most animal species, the female has a slightly lower hematocrit and red cell volume than does the male, but of all the animals studied, the chicken shows the most remarkable difference between the male and female, the female having approximately half the hematocrit of the male and half the cell mass. Blood volumes of normal male and female chickens were measured and the effects of administered testosterone on the female were studied. It was found that following the administration of intramuscular testosterone, the hen showed a remarkable increase in the hematocrit values approaching that of the male. This was confirmed by red cell mass determinations by Cr51 as well.

The electrophoretic patterns of hemoglobins of various species of animals were studied by paper electrophoresis. Among the results of interest was the finding that some birds have as many as 3 different hemoglobins, some turtles have 2, and one species of turtle as many as 3 different types of hemoglobin; a few sheep had 2 different types of hemoglobin, and a few horses. The remainder of the animals studied showed a single hemoglobin although the rates of mobility,were different, being much slower in the case of the amphibians and reptiles, snakes, the turtle showing a faster rate of migration than the snake.

Further studies on the use of sodium chromate-51 as a measure of in vivo life span were productive. The question was explored as to whether or not there was any deleterious effect of washing the cells once or twice with saline before transfusion. Accordingly, in 4 normal individuals, the red cell survival of washed and unwashed tagged cells was measured, and there was found to be no significant difference between washed and unwashed cells.

A table was prepared for correcting the results of chrome-tagged red cell transfusion so that they may be expressed in more physiological terms and be more representative of what is actually happening to the transfused red cell. The amount of error involved in this correction was also determined.

The use of sodium chromate tagged red cells as a measure of gastrointestinal blood loss was also evaluated. Chrome-tagged blood was given orally to 3 normal subjects. It was found that 90 per cent or more of the tagged blood given in this fashion was recovered in the stools and that virtually none of the administered sodium chromate in the form of tagged red cells was reabsorbed. In one instance, plasma tagged with chromic chloride was given to a normal subject orally. Again, it was found that 90+ per cent of the administered chromic chloride was excreted in the stools. It was further found that the chromium was excreted in the stool in a non-dialyzable form. The use of intravenously administered sodium chromate-tagged blood was then applied in 3 cases which ultimately proved to have an anemia of blood loss from the gastrointestinal tract. In these 3 patients, it was found to be of interest to quantitate the exact amount of blood loss in the stool during a given period. In one instance, a patient lost as much as 1000 cc. of blood via the stool over a 4-day period. This occurred without a change in the normal color of the stool. The sensitivity of the chromium method for assessing blood loss in the bowel was compared to the Ham Test. The Ham Test was found to be as sensitive as the chromium method but somewhat more erratic, due to the fact that the Ham Test as conventionally performed is based on a very small sample of stool and hence negative results may be obtained where actually a significant amount of blood is present in the stool. The data obtained on normal subjects would indicate that a normal subject does not excrete more than 1-5 ml. of blood during a 24-hour period; most normals excreted closer to 1 ml.

The rate of red cell destruction in the idiopathic hyperbilirubinemia was studied. No previous studies on the rate of red cell destruction in this disease exists, and it was possible that the reason for the increased bilirubin was due to increased cell destruction. The study of 3 patients proved this, however, not to be the case, since the rate of chromium tagged autotransfused cell destruction was within normal limits.

Significance to NIAMD Research: The additional data on the nature and rate of elution of Cr^{51} from circulating red cells *in vivo* was of practical importance in the employment of the Cr^{51} technique for the measurement for the survival of the red cell *in vivo*. It does now appear that this technique is of practical value in the study of anemia provided that certain limitations which were pointed out in both studies are recognized. The use of Cr^{51} tagged cells for the quantitative estimation of the rate of blood loss from the stool in patients either suspected or proved gastrointestinal bleeding should prove of practical value in the management of such patients. Indeed, the findings obtained by this method led to more vigorous pursuit and measures for correction of the lesion when the magnitude of the blood loss was apparent, as measured by Cr^{51}. Other studies, such as the Ham Test and the clinical observations of the patient led the physicians to greatly underestimate the seriousness of blood loss. On the other hand, it should be pointed out that the use of Cr^{51} as a measure of blood loss in the stool is only

adjunct to the careful clinical management and employment of all the current tests for the study and diagnosis of blood loss in the stool. It is certainly not a substitute for any one of the currently recognized techniques for this study. For example, the Cantor tube, a technique by no means new, was found to be a very valuable adjunct in the localization of a site of bleeding in the gastrointestinal tract.

The project studying the anemia of rheumatoid arthritis was brought to a conclusion and with the presence of adequate normal data, it was established that the major defect in anemic patients with active rheumatoid arthritis is the failure of adequate blood production and not to an increased rate of destruction. The mechanism of the failure of adequate blood production is completely unknown. Studies already performed indicate that it is not due to iron deficiency since normal readily miscible iron pools were found in patients studied, despite the low serum iron. Further studies of the comparative rates of red cell survival in various species of animal would appear to be of value in gaining further insight in factors underlying red cell turnover. It would be highly desirable for such studies to be done by a single technique and done simultaneously on animals whose metabolic rates are measured in the same laboratory.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-104-c
SERIAL NUMBER

12. Research
BUDGET ACTIVITY

13. COOPERATING UNITS: Dr. Milton Silverman's laboratory in the Laboratory of Biochemistry and Nutrition of NIAMD.

14. COMPLEMENTARY OR PARALLEL RESEARCH IN PHS: Studies conducted in the laboratory of Dr. Paul Altland on the Survival of the Red Cell in the Turtle and Duck complement and parallel certain other studies described in this project. Certain studies of chromium survival of the red cell in dogs done in the laboratory of Dr. Frederick Stohlman complement and parallel certain aspects of the project herein described.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

16. PUBLICATIONS FROM THIS PROJECT:

The Anemia of Rheumatoid Arthritis. F. G. Ebaugh, Jr., R. E. Peterson, G. P. Rodnan, and J. J. Bunim. Med. Clinics of North America, 39:49, 1955.

17. No honors or awards.
HONORS OR AWARDS

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
 INSTITUTE

2. Arthritis and Rheumatism
 LABORATORY OR BRANCH

3. Clinical Investigations
 SECTION

4. _____

5. NIAMD-105-c
 SERIAL NO.

6. Folic Acid Metabolism in Anemia
 PROJECT TITLE

7. Franklin G. Ebaugh, Jr., M. D.
 PRINCIPAL INVESTIGATOR

8. Milton Silverman, M. D.
 OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The purpose of this project was to discover any defect in the excretion of the labile citrovorum factor in the anemia of bone marrow failure.

Methods Employed: 24 hour urines were collected from patients, each sample being frozen immediately after voiding. The amount of folic acid was then analyzed by bioassay, utilizing Streptococcus fecalis and the citrovorum factor both stabile and labile were analyzed utilizing the Luconostoc citrovorum. These bioassays were performed by Dr. Milton Silverman. In order to have suitable controls, the excretion of the labile factor and other factors influencing its excretion were studied in patients with various types of anemia and normal controls.

Patient Material:		No.	Average Stay in Days
Admissions:	Adult males	10	54
	Adult females	8	7
	Children	0	
Outpatient:	No. of patients	0	
	No. of visits	0	

Major Findings: During the project it was found necessary to critically evaluate factors other than those directly involving the metabolism of folic acid, which influenced the quantitative amount of labile factor present in the urine. Previous efforts had indicated that Vitamin C administered to animals increased the amount of labile citrovorum factor excreted. This was confirmed but the implication

that Vitamin C had some direct involvement in the metabolism and conversion of folic acid to the labile citrovorum factor could not be definitely proved, since Vitamin C was found to exert a protective effect on the preservation of excreted labile citrovorum factor once present in the urine. In addition it was found that the pH of the urine was important in determining the amount of labile citrovorum factor ultimately recovered, there being greater amounts present when the urine was acid than when the urine was alkali, and indeed, it could be shown that acidification of the urine by ammonium chloride caused in some patients as great a rise in the labile citrovorum factor as did the administration of Vitamin C. Several patients with anemia of marrow failure were studied and found to excrete a normal amount of labile factor. Many other types of anemia were studied, such as hemolytic, of which sickle cell was representative, leukemia, anemia of infection, and the anemia of rheumatoid arthritis. In none of these various anemic states was there a significant alteration in the amount of labile, stabile, citrovorum factor of folic acid found.

Significance to NIAMD Research: The major significance of this project was a negative one, namely, that no metabolic defect as determined by urinary excretion studies could be determined with respect to the excretion of labile citrovorum factor in various anemias. Important for future studies was the finding that careful control of urinary pH must be made in order to correctly evaluate the amount of labile citrovorum factor excreted. The role of Vitamin C with respect to folic acid metabolism remains unsettled. Evidence was produced in this project to indicate that one did not necessarily have to postulate the direct effect of Vitamin C on the increase of excretion of labile citrovorum factor, as far as conversion of folic acid to this compound is concerned. Direct evidence was obtained that at least one of the reasons why labile citrovorum factors increased in the urine is due to the protective action of Vitamin C while the urine is in the bladder and after excretion.

Proposed Course of Project: It is proposed that the project be terminated at the end of calendar year 1955.

Note: Dr. Milton Silverman served as a senior principle investigator in another phase of this project, namely, the isolation and characterization of the chemical nature of the labile citrovorum factor as excreted in normal and diseased human urine.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-JC5-c
SERIAL NUMBER

12. Research
BUDGET ACTIVITY

13. NO COOPERATING UNITS

14. NO PARALLEL RESEARCH IN PHS

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

16. NO PUBLICATIONS

17. NO HONORS OR AWARDS

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Arthritis and Rheumatism
LABORATORY OR BRANCH

3. Clinical Investigations
SECTION

4. _____

5. NIAMD-106-c
SERIAL NO.

6. Iron Metabolism in Man, in Health and Disease
PROJECT TITLE

7. Ralph E. Peterson, M.D.
PRINCIPAL INVESTIGATOR

8. G. Rodnan, F. Ebaugh, Jr., M. Bollier, T. Clemens, E. Marsden
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Determination of the turnover rate of iron in the normal male and female, and in patients with various hematological disorders.

Methods Employed: Iron turnover rate is determined by the use of radioactive iron.

Patient Material:

		No.	Average Stay in Days
Admissions:	Adult males	15	20
	Adult females	17	22
	Children	0	
Outpatient:	No. of patients	8	
	No. of visits	25	

Major Findings: Iron turnover rate studies have been completed in 20 normal male and 20 normal female subjects. Female subjects have shown the following: miscible iron pool = 30-77 µg/kilo; iron turnover rate = .28-.86 mg/k/day; milligrams of iron turned over for hemoglobin production = .18-.50 mg/k/day, or a mean of approximately 15 mg. of iron turned over per liter of red cells. Male subjects have shown: miscible iron pool of 28-133 µg/kilo; iron turnover rate of .41-1.1 mg/k/day, iron turnover rate for hemoglobin production of .32-.75 mg/k/day, with a mean of approximately 18 mg. of iron turned over per liter of red cells.

Further studies of iron turnover rate in various hematological disorders have failed to delineate the mechanism that might explain the poor correlation of the iron turnover rate with the rate of hemoglobin production.

Significance to NIAMD Research: The studies on iron turnover are directed toward an evaluation of this method as a simple procedure for investigating hemoglobin synthesis in vivo.

Proposed Course of Project: Plans are to discontinue this project unless we can find an explanation for the apparent discrepancy between the iron turnover rate and the rate of hemoglobin production.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-106-c
SERIAL NUMBER

12. Research
BUDGET ACTIVITY

13. Georgetown University Hospital
COOPERATING UNITS

14. National Heart Institute
PARALLEL RESEARCH IN PHS

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

16. PUBLICATIONS FROM THIS PROJECT:

The Anemia of Rheumatoid Arthritis. F. G. Ebaugh, Jr., R. E. Peterson, G. P. Rodnan, J. J. Bunim. Med. Clinics of North America, 39:489, 1955.

Hematological Observations on Sickle Cell Anemia Patients Sustained at Normal Hemoglobin Levels by Multiple Transfusions. H. Chaplin, H. G. Keitel, R. E. Peterson, In press.

17. No honors or awards.
HONORS AND AWARDS

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Arthritis and Rheumatism
LABORATORY OR BRANCH

3. Clinical Investigations
SECTION

4. _____

5. NIAMD-107-c
SERIAL NUMBER

6. Metabolism of Adrenocortical Steroids
PROJECT TITLE

7. Ralph E. Peterson, M.D.
PRINCIPAL INVESTIGATOR

8. C. Pierce, M. Bollier, A. Karrer
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

1. Pharmacology and physiological disposition of various adrenocortical steroids in man.

2. Metabolic transformations of these steroids in man.

3. The turnover rate of endogenous hydrocortisone and corticosterone in man.

4. The development of newer improved methods for the determination of corticosteroids and their metabolites in biological fluids.

Methods Employed: Pharmacological quantities of steroids and steroids labeled with Carbon^{14} have been used to carry out many of these studies. Improved methods have been devised for the assay of steroids in micro quantities.

Patient Material:

		Number	Average Stay in Days
Admissions:	Adult males	20	15
	Adult females	16	12
	Children	0	
Outpatient:	No. of patients	5	
	No. of visits	10	

Major Findings: Utilizing trace quantities of intravenously administered hydrocortisone-4-C^{14}, we have determined the magnitude of the miscible pool of hydrocortisone in the normal subject to be 1.1 to 2.4 mg. This pool of hydrocortisone has been found to be distributed in an apparent volume of 8 to 17 liters. Calculations of turnover rate of endogenously synthesized hydrocortisone have ranged from 17 to 29 mg. per day in 9 normal subjects. Following maximal adrenocorticotropin stimulation, the miscible pool of hydrocortisone increased to 10 mg., and the turnover rate increased to 154 mg. per day in the normal subject. Following oral administration of delta 1-cortisone (prednisone), a marked decrease in both pool size and hydrocortisone turnover rate was demonstrated. The miscible pool and turnover rate of hydrocortisone have been found to show a diurnal variation.

In cirrhosis of the liver and myxedema, the miscible pool is approximately normal in size; however, the turnover rate of hydrocortisone is reduced by one-third to one-fifth of normal. In thyrotoxicosis, the miscible pool is also normal; however, the turnover rate has been found to be increased 2-4 times normal.

The studies on the metabolic transformation of hydrocortisone in vivo have demonstrated the presence of two metabolites that have previously not been reported--20β-hydroxy hydrocortisone and 6β-hydroxy hydrocortisone.

Further improvements and additional evaluation studies have been completed on the plasma hydrocortisone method and the urinary corticosteroid and 17-ketosteroid methods.

An isotopic dilution procedure has been developed for the determination of plasma hydrocortisone in various clinical conditions where there is some doubt about the specificity of the standard plasma hydrocortisone procedure. This has proved especially revealing in diabetic acidosis where it has been reported that the level of hydrocortisone in the plasma is markedly elevated. With this technique, we have been able to demonstrate that this is a spurious elevation and that the true plasma hydrocortisone level is normal. Also, in one study, this was further substantiated by the finding of a normal pool size and normal turnover rate of hydrocortisone.

A new method for the determination of plasma corticosterone has been developed that is much more selective than the previously published method. This also is an isotopic dilution method. Results to the present would indicate that the normal plasma corticosterone level ranges from 0.4-1.4 μg%. This is a value that is considerably lower than previously published figures. Following ACTH stimulation, the normal level increases 6-8 fold and following administration of various steroids that suppress completely adrenal hydrocortisone output, the plasma corticosterone levels become zero.

Various studies on the effects of stress and various drugs on the pituitary adrenal axis are being carried out in collaboration with the National Institute of Mental Health. Preliminary results would indicate that under the experimentally induced stress of piromen, a very marked increase in hydrocortisone production results. This effect can be abolished by the prior administration of aspirin.

Significance to NIAMD Research: The studies on physiological disposition and pharmacology are important in regard to the treatment of patients with steroids. The studies on turnover rate should make it possible for the first time to determine the actual production of hydrocortisone and corticosterone by the adrenals in various disease states and in normal subjects. The studies on methodology are necessary to make it possible to more reliably carry out all of these studies.

Proposed Course of Project:

1. Additional data on the pharmacology and physiological disposition of corticosterone in man.

2. Additional data on the plasma corticosterone levels in various diseases, and other various experimental conditions.

3. Determination of the turnover rate and miscible pool of corticosterone in normals and in patients.

4. Turnover rate and miscible pool of hydrocortisone and corticosterone in pregnancy.

5. Further studies on the isolation and identification of the metabolites of hydrocortisone in the urine and perhaps qualitative and quantitative studies on the patterns of these metabolites in the urine in normal and disease states.

6. Evaluation of the relative potency of various adrenocortical steroids and their synthetic analogues with regard to adrenal suppression and the possible correlation of the degree of suppression with the anti-inflammatory action of these steroids.

7. Development of enzyme methods for assay of various adrenocortical steroids.

8. Studies on the conjugates of adrenal steroids.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-107-c
SERIAL NUMBER

12. Research
BUDGET ACTIVITY

13. National Institute of Mental Health
COOPERATING UNITS

14. None
PARALLEL RESEARCH IN PHS

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

16. PUBLICATIONS FROM THIS PROJECT:

Physiological Disposition and Metabolic Fate of Hydrocortisone in Man. R. E. Peterson, J. B. Wyngaarden, S. L. Guerra, B. B. Brodie, and J. J. Bunim. J. Clin. Investigation, 34:1779, 1955.

An Improved Method for Assaying the Steroidogenic Potency of ACTH. G. W. Liddle, J. Richard, and R. E. Peterson, J. Biol. Chem., In press.

The Miscible Pool and Turnover Rate of Hydrocortisone in Man. R. E. Peterson, and J. B. Wyngaarden. J. Clin. Investigation, In press.

17. No honors or awards.
HONORS AND AWARDS

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Laboratory of Pathology and Histochemistry
LABORATORY OR BRANCH

3. Clinical Investigations
SECTION

4. _____

5. NIAMD-108-c
SERIAL NUMBER

6. Studies on the Histopathology of the Synovial Membrane in Various Types of Arthritis
TITLE OF PROJECT

7. Leon Sokoloff
PRINCIPAL INVESTIGATOR

8. NONE
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Little information concerning the pathological changes in the articular structures in many types of arthritis and rheumatism is available. This histological study is designed to elucidate the nature of certain arthritic disorders, improve diagnosis and follow the effects of various therapies on the disease processes.

Methods Employed: Open biopsy of the knee joint is carried out under local anesthesia. Appropriate anatomical studies are made at necropsy in a smaller proportion of cases.

Patient Material:

Admissions:	No.	Average Stay in Days
Adult males	7	6
Adult females	4	12
Children male	0	
Children female	0	

Outpatient:	
Number of patients	0
Number of visits	0

Major Findings:

1. Chronic synovitis has been found to be a common lesion of scleroderma. Although joint pains are common in this disease and its onset is often confused with rheumatoid arthritis, the joint changes have not been described previously.

2. A syndrome of polyarthritis associated with Boeck's sarcoid has been recognized in recent years; frequently regarded as a variant of rheumatoid arthritis, the biopsy in one case clearly demonstrates the articular lesion to be sarcoid rather than rheumatoid.

3. Gouty arthritis is ordinarily easily distinguished from rheumatoid arthritis. In perhaps as many as 10% of rheumatoid patients, hyperuricemia is present. Biopsy of several such patients has demonstrated that they do in fact have gout rather than rheumatoid arthritis, while the diagnosis of rheumatoid arthritis rather than gout is established in others.

Significance to NIAMD Research: The findings described have clarified the character of three types of arthritic disorder that previously have been unrecognized or confused with rheumatoid arthritis. In addition, the effect of several new steroid preparations on the synovial membrane has been followed. It has been found that none of these has extirpated the pathological process although in several cases with severe rheumatoid synovitis biopsies done before and during the fourth week of prednisone therapy there was a marked subsidence of the inflammation process as a result of steroid administration.

Proposed Course of Project: Similar biopsies are to be performed to expand these observations and to extend them to other types of arthritis as well.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-108-c
SERIAL NUMBER

12. Research
BUDGET ACTIVITY

13. No cooperating units.
COOPERATING UNITS OF PHS

14. No parallel research in PHS.
PARALLEL RESEARCH IN PHS

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

16. PUBLICATIONS FROM THIS PROJECT

Some aspects of the pathology of collagen diseases. L. Sokoloff, Proc. N. Y. Path. Soc., Bull. N. Y. Acad. Med., in press.

Observations on the Use of Prednisone in Patients with Progressive Systemic Sclerosis (Diffuse Scleroderma). G. P. Rodnan, et al., Annals Int. Med., January, 1956.

17. No honors or awards.
HONORS AND AWARDS

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Clinical Investigation - Arthritis & Rheumatism Branch
LABORATORY OR BRANCH

3. _____
SECTION

4. Bethesda
LOCATION

5. NIAMD-109-c
SERIAL NUMBER

6. Enzymatic Mechanisms of Corticosteroid Metabolism
PROJECT TITLE

7. Gordon Tomkins, M. D., Ph.D; Kurt J. Isselbacher, M. D.
PRINCIPAL INVESTIGATORS

8. Julius Axelrod
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: It is known that corticosteroids are metabolized in the body to inactive "tetrahydro" derivatives. These compounds are then conjugated and excreted to a significant extent in the form of their glucuronides. We have attempted to study the mechanisms and pathways involved in this process by isolating the enzymes and determining the cofactors required for these reactions.

Methods Employed: We have used liver from mammalian sources (mostly guinea pig and rat), converted them to homogenates and then to various cell free preparations. Enzymes were studied and isolated by resorting to techniques of differential ultra-centrifugation, fractionation with ammonium sulfate and adsorption on, and elution from, various gels. Steroids used for the study included primarily the derivatives of cortisone and also the newer delta-I cortisone (prednisone).

Patient Material: None

		No.	Average Stay Days
Admissions:	Adult males Adult females Children male Children female	None	None
Outpatient:	Number of Patients Number of visits	None	

Major Findings: During the course of the year, four different and distinct enzymes were identified and isolated and their various cofactor requirements determined.

The results have been summarized in the following table:

Reaction Catalyzed	Enzyme	Cofactor Required
1. Delta-1-cortisone ⟶ cortisone	Cortisone Dehydrogenase	Reduced TPN
2. Cortisone ⟶ Dihydrocortisone	Dihydrocortisone Dehydrogenase	Reduced TPN
3. Dihydrocortisone ⟶ Tetrahydrocortisone	3α-Hydroxysteroid Dehydrogenase	Reduced TPN or Reduced DPN
4. Tetrahydrocortisone ⟶ tetrahydrocortisone glucuronide	Glucuronosyl Transferase	Uridine Diphosphate Glucuronic Acid (UDPGA)

The final reaction (4) elucidates the mechanism of steroid glucuronide formation, and, as indicated, requires the presence of an "active form" of glucuronic acid, viz., uridine diphosphate glucuronic acid (UDPGA). The existence of uridine nucleotides has only recently been recognized and this is one of the first demonstrations of its role in the mammalian organism. In a similar fashion, we have achieved the glucuronide conjugation of other compounds such as L-thyroxine, estradiol, testosterone and tetrahydro-hydrocortisone.

Recently studies have also been underway to study a reaction in which etiocholane 3,17-dione is converted to its isomer, androstane 3,17,dione.

IAMD-109-c
ERIAL NO.

Significant progress has been made in isolating and purifying the enzyme involved.

Significance to NIAMD Research: These enzymatic studies integrate well with the overall studies of corticosteroid metabolism in the Institute. The findings provide for the first time details about the fundamental pathways of steroid metabolism which are necessary prerequisites to an understanding of the mechanism of action of these important compounds normally and in disease states. Furthermore, from these enzymatic studies it appears that there may soon be available specific tests to determine blood levels of the various corticosteroids.

Proposed Course of Project: 1. It is planned to pursue the isolation of the isomerase enzyme described above and to evaluate its significance. 2. Studies are underway to apply the specificity of the enzymes isolated to the determination of various blood steroid levels. 3. Plans are underway to elucidate the hitherto obscure mechanism of estrogen formation, with special reference to the manner in which the aromatic ring of the estrogens is formed.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-109-c
SERIAL NUMBER

12. BUDGET ACTIVITY

Research

13. No cooperating units.

14. No parallel research in the Public Health Service.

Honors, Awards and Publications

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Enzymatic Formation of Corticosteroid Glucuronides, - K. J. Isselbacher and J. Axelrod, J. Am. Chem. Soc., 77: 1070, 1955.

A Mammalian 3α-Hydroxysteroid Dehydrogenase, - G. Tomkins, J. Biol. Chem., In press.

17. No honors or awards.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Clinical Investigation - Arthritis & Rheumatism Branch
LABORATORY OR BRANCH

3. _____ 4. Bethesda
SECTION LOCATION

5. NIAMD-110-c
SERIAL NUMBER

6. Studies of Purine Metabolism in Gout
PROJECT TITLE

7. Seegmiller, J. E.; Laster, Leonard; Wyngaarden, J. B.
PRINCIPAL INVESTIGATORS

8. Liddle, Lois; Blair, Alberta; Love, Ethel
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To gain understanding of clinical gout by studying uric acid biosynthesis and excretion in normal and gouty subjects, and by investigating the mechanism of action of drugs which influence these factors.

Methods Employed: The incorporation of isotope from orally administered labeled precursors into various purine excretion products was studied in normal and gouty subjects. Uric acid excretion in patients with various stages of gout, and the effect of drugs on this excretion were investigated. The serum and urine of patients were assayed with enzymatic and chromatographic methods for compounds related to purine metabolism. Determinations were made of the magnitude of the miscible pool of uric acid in human subjects using uric acid-2-C^{14}. Patients suffering from acute gouty arthritis were treated with intravenously administered colchicine and detailed observations of the response to this medication were made.

Patient Material:		No.	Average Stay Days
Admissions:	Adult males	36	29
	Adult females	8	22
	Children male	None	
	Children female	None	
Outpatient:	Number of patients	28	
	Number of visits	74	

Major Findings: Our previous studies of the incorporation of 4-amino-5-imidazole carboxamide-4-C^{13} (AIC-C^{13}) into uric acid in normal subjects have been extended to patients with gout. Two gouty patients who incorporated glycine-N^{15} into uric acid in a manner similar to normal subjects showed a pattern of incorporation of AIC-C^{13} into uric acid that also closely resembled normal subjects in that there were two pathways for this incorporation. There was some evidence to suggest an increased magnitude of incorporation in the gouty patients. As in the normal subjects, AIC depressed the de novo synthesis of uric acid from glycine.

One patient suffering an attack of acute gout incorporated glycine-N^{15} into uric acid in a manner and quantity similar to normal subjects.

Following oral administration of glycine-1-C^{14}, the isotope concentration in urinary purines, (uric acid, xanthine, hypoxanthine, adenine), were determined and compared over 8-14 day periods in normal and gouty subjects. In both types of subject, uric acid was more highly enriched than any of the other three purines at all points in time, except that hypoxanthine enrichment exceeded that of uric acid on the first day. From the shapes of the various curves, it would appear that in both normal and gouty subjects there is evidence that uric acid is synthesized via hypoxanthine by a rapid pathway, and also via adenine by a slower pathway. In one gouty "overproducer", evidence suggested that the early pathway involving hypoxanthine was utilized to a greater extent than in the control subject.

In treating acute gout with colchicine administered intravenously, the following advantages have been noted in some patients as compared with oral administration: A more rapid response with lower total dosage, fewer side effects, and, at times, a satisfactory response in patients whose attacks had not responded to orally administered colchicine.

Significance to NIAMD Research: Using AIC-C^{13} we have shown that one class of gouty patients is similar to normal subjects in the pattern of urate biosynthesis. This agrees with previous studies using glycine-N^{15} in this class of patients. In addition, we have not detected any effect of acute gout on urate biosynthesis from glycine-N^{15}.

The findings with glycine-1-C^{14} to date are in agreement with the hypothesis that there are two major routes of urate synthesis in man, one a rapid shunt pathway, the other a slower, quantitatively more important pathway involving nucleic acids as intermediates. The data obtained with glycine-C^{14} suggest that the rapid shunt pathway of urate synthesis may involve hypoxanthine (free or combined with ribose $\pm$ phosphate) as an intermediate. They further raise the question whether urate may also be synthesized via pathways not involving free hypoxanthine and xanthine as intermediates.

The evidence obtained thus far suggests that the use of colchicine administered intravenously may have definite advantages over the oral route of administration for the treatment of acute gout.

Proposed Course of Project: A study is in progress in gouty patients who excrete abnormally large amounts of uric acid to determine the pattern of incorporation of AIC-C^{13} into uric acid. Evidence from previous work with glycine-N^{15} suggests that the results obtained in this study will differ from those obtained with the gouty patients studied so far.

Other diseases associated with disorder of purine metabolism will be studied in similar fashion, using AIC-C^{14} in trace quantities. Further studies will be made of acute gout, its precipitating factors, associated changes of urate metabolism and its therapy.

Studies are in progress of the enzymatic degradation of colchicine by tissue homogenates.

Studies of rates and extents of precursor incorporation into urinary purines will be extended to include precursors other than glycine, such as AIC and purines (e.g. hypoxanthine, inosine, etc.). Also, alternate pathways of urate synthesis involving preliminary C-8 oxidation of amino-hydroxypurines, will be studied in vitro.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-110-c
SERIAL NUMBER

12. Budget Activity: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S)(ITEM 10)

Section on Intermediary Metabolism, LBN - NIAMD

14. No parallel research in Public Health Service

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Incorporation of 4-Amino-5-imidazolecarboxamide-4-C^{13} into Uric Acid in the Normal Human, J. E. Seegmiller, Leonard Laster and DeWitt Stetten, Jr., - J. Biol. Chem. 216:653, 1955.

The Effect of Phenylbutazone on Uric Acid Metabolism in Two Normal Subjects, J. B. Wyngaarden, - J. Clin. Investigation, 34:256, 1955.

Uric Acid, J. B. Wyngaarden, - Cyclopedia of Medicine, Surgery, Specialties, F. A. Davis Co., Philadelphia, 1955, p 341-355.

Plasma Glutanine and Oxypurine Content in Patients with Gout, S. Segal and J. B. Wyngaarden, - Proc. Soc. Exper. Biol. Med., 88:342, 1955.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases

2. Clinical Investigations
LABORATORY

3. Clinical Endocrinology
BRANCH

4. Bethesda
LOCATION

5. NIAMD-111-c
SERIAL NUMBER

6. Studies of the Mechanism of Action of Antithyroid Drugs
PROJECT TITLE

7. Joseph E. Rall, M. D. and Jan Wolff, M. D.
PRINCIPAL INVESTIGATORS

8. Jane Heslin
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Title of Project: Studies of the Mechanism of Action of Antithyroid Drugs.

Objectives: To test certain drugs for their action in inhibiting organic binding of the thyroid and inhibiting or enhancing iodide concentration by the thyroid.

Methods Employed: 1) The uptake of radioiodine by the thyroid of rats under various experimental procedures.
2) The use of isolated slices of thyroid tissue in acute experiments with drugs and I^{131}.

Patient Material: None.

Major Findings: Drugs tested were cysteine (I), 2-mercaptoethylamine (II) and 2,2'dithiobis (ethylamine) 2HCl (III). There was no effect of I on iodide concentration in the intact animal. Both II and III increased the iodide concentration in the thyroid within 2 hours as measured in the thyroid gland blocked with thiouracil. The ratio of activity in the thyroid (in these animals entirely iodide) compared to the activity in the blood is the T/S. The T/S was increased from 20 to 60 by either II or III. In addition, in the animals with unblocked thyroids, i.e., those not receiving thiouracil, the total amount of I^{131} accumulated in the course of 6 hours was decreased

when these drugs had been administered. These results suggest that both II and III are antithyroid drugs in that they inhibit organic binding by the thyroid. In addition they cause an acute increase in the iodide concentrating mechanism of the thyroid. The effect of these drugs was investigated in nephrectomized rats, with inconclusive results. A dose response curve for II showed a perceptible increase in the T/S at a single dose level of 18 mg/Kg and a progressive increase in effect up to the maximum dose employed, which was 180 mg/Kg.

In hypophysectomized rats given TSH there was no conclusive effect of these drugs on the T/S. In hypophysectomized rats not given TSH and on either a low iodine diet or a normal diet, there again appeared to be no effect on the T/S from the administration of II and III.

Significance to NIAMD Research: It is hoped that the very marked effect of these drugs on increasing the T/S will give some insight into the mechanism by which iodide is concentrated. These compounds also represent a new class of antithyroid drugs and are of interest from this standpoint.

Proposed Course of Project: Further studies will be done with hypophysectomized animals in an attempt to explain why these drugs so far have been without effect in these animals. Additional related drugs will be investigated, the effect of long-term administration of these drugs on the thyroid will be investigated, and further work with *in vitro* systems is planned.

Analysis of NIH Program Activities

10. NIAMD-lll-c
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. No cooperating units.

14. No parallel research in the Public Health Service.

16. No publications from this project during calendar year 1955.

17. No honors and awards relating to this project during calendar year 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases

2. Clinical Investigations
LABORATORY

3. Clinical Endocrinology
BRANCH

4. Bethesda
LOCATION

5. NIAMD-112-c
SERIAL NUMBER

6. Effect of Thyroxine on Enzyme Systems in vitro
PROJECT TITLE

7. Jan Wolff, M. D.
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Title of Project: Effect of Thyroxine on Enzyme Systems in vitro.

Objectives: It is hoped that this in vitro approach may offer clues regarding the mechanism of action of thyroxine.

Methods Employed: Crystalline or purified enzyme preparations are allowed to act on suitable substrates in the presence of DPN and the initial rates of the reactions are measured with or without thyroxine analogues. Up to the present this has involved alcohol and malic dehydrogenases. The rate of appearance of DPNH is measured by UV absorption.

Patient Material: None.

Major Findings: L-thyroxine ($3.3 \cdot 10^{-5}$ Molar) produces at least 60%, and at 10^{-4} Molar $\geq$ 90% inhibition of DPN reduction. Lineweaver and Burke plots suggest that the inhibition is competitive in type with respect to both malate and DPN. Thyroxine produces no significant shift in the UV absorption spectra of DPN or DPNH in the concentrations employed. L-triiodothyronine appears to be as active as thyroxine, whereas triiodothyroacetic acid, D-thyroxine, D,L-tetrachlorthyronine, D,L-3,5-diiodo-3',5'-dimethylthyronine, and butyl 4-hydroxy-3,5-diiodobenzoate show less effect (about 50% inhibition at 10^{-4} Molar). DL-thyronine, 3,5-diiodothyronine,

L-diiodotyrosine, DL-tetranitrothyronine, DL 3,5-diiodo-3',5'-dinitrothyronine and 2,4-dinitrophenol show insignificant or no inhibition at 10^{-4} Molar concentration.

L-thyroxine also interferes with the oxidation of ethanol by yeast alcohol dehydrogenase in the presence of DPN. At thyroxine concentrations of $1 \cdot 10^{-4}$ to $5 \cdot 10^{-5}$ L-thyroxine produced about a 50% inhibition in DPN reduction. D-thyroxine appears to be as active an inhibitor while L-triiodothyronine and triiodothyroacetic acid are somewhat stronger on a molar basis.

Significance to NIAMD Research: The ultimate goal in this study is to shed light on the mechanism of action of the thyroid hormones. Such knowledge could not help but be of use in the understanding and treatment of thyroid dysfunction.

Proposed Course of Project: The in vitro studies will be extended to other dehydrogenases and certain other isolated enzyme systems in order to establish: 1) The nature of the competition involved, 2) the possibility of a thyroxine effect on the dissociation of enzyme-DPN or enzyme-substrate complexes, 3) metal complexing effects of thyroxine in certain metallo-enzymes.

10. NIAMD-112-c
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. No cooperating units.

14. No parallel research in the Public Health Service.

16. No publications from this project during calendar year 1955.

17. No honors and awards relating to this project during calendar year 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases

2. Clinical Investigations
LABORATORY

3. Clinical Endocrinology
BRANCH

4. Bethesda
LOCATION

5. NIAMD-11?-c
SERIAL NUMBER

6. Studies on the Specific Thyroxine-binding Protein of Serum
PROJECT TITLE

7. Jacob Robbins, M. D.
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Title of Project: Studies on the Specific Thyroxine-binding Protein of Serum.

Objectives: 1) To measure the thyroxine-binding capacity of the specific thyroxine-binding protein of human serum in normal and diseased states, 2) to further characterize this protein, and 3) to evaluate its function in thyroid physiology.

Methods Employed: Zone electrophoresis, chromatography, and other chemical procedures, employing radioactive iodine as an indicator.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	2	16
	Adult females	7	22
	Children	0	
Outpatient:	Number of patients	8	
	Number of visits	26	

Major Findings: A modified technique for zone electrophoresis has been developed which prevents contamination of TBP-bound thyroxine with albumin-bound TBP. This permits the direct measurement of the thyroxine-binding capacity of TBP in serum. In normal serum, this value varies from .16 to .24 μg per ml (mean = .19) and is the same for men and women. In late pregnancy, TBP can bind from .31 to .53 μg of thyroxine per ml (mean = .41). This change in thyroxine-binding capacity may be the cause of the elevated serum protein bound iodine which occurs in pregnancy.

Significance to NIAMD Research: The role of the specific thyroxine-binding protein in thyroid physiology is not understood. This study represents the beginning of an attempt to evaluate its function and its possible participation in certain abnormal states.

Proposed Course of Project: To determine the thyroxine-binding capacity of TBP in a number of abnormal states and to attempt to determine the mechanism by which any abnormalities may occur (e.g., in pregnancy).

Analysis of NIH Program Activities

10. NIAMD-113-c
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. No cooperating units.

14. No parallel research in the Public Health Service.

16. No publications from this project during calendar year 1955.

17. No honors and awards relating to this project during calendar year 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases

2. Clinical Investigations
LABORATORY

3. Clinical Endocrinology
BRANCH

4. Bethesda
LOCATION

5. NIAMD-114-c
SERIAL NUMBER

6. Studies of Pentose Metabolism in Man
PROJECT TITLE

7. Stanton Segal, M.D. and James B. Wyngaarden, M.D.
PRINCIPAL INVESTIGATORS

8. Joseph Foley and Alberta Blair
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Title of Project: Studies of Pentose Metabolism in Man.

Objectives: To obtain information on the distribution, excretion and metabolism of pentoses in man, and the effects of insulin thereon.

Methods Employed: Pentoses D-xylose, D-arabinose, L-arabinose were infused intravenously into control and diabetic subjects, and patients with liver disease. The blood levels and urinary excretion of pentose were determined, and the effect of pentose infusions on blood levels of glucose, pyruvate, lactate and phosphate were measured. The effect of intravenous insulin on the hemilogarithmic disappearance rate of pentoses was studied.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	8	56
	Adult females	5	18
Outpatient:	Number of patients	1	
	Number of visits	6	

Major Findings: Pentoses disappear from blood at rates proportional to concentration, with biologic half times ranging from 44 to 96 minutes. Urinary recoveries ranged from 27-60%. No significant changes occurred in blood pyruvate and lactate levels, but glucose levels increased an average of 20 mg% with D-xylose and L-arabinose. Phosphate levels showed a slight early fall followed by a late rise. When insulin was given a marked decrease of blood level of D-xylose and L-arabinose resulted, which was maximal in 30 minutes, and represents a change of 22-33% from the concentration anticipated by extrapolation of the initial hemilogarithmic decay curve. Studies with insulin have not yet been conducted in diabetics or patients with liver disease, but pentose infusions in such patients have shown no major difference in blood disappearance rate or urinary recovery of D-xylose in the diabetic subject (whether on or off insulin), whereas the disappearance rates of D-xylose were considerably slower in patients with cirrhosis.

Significance to NIAMD Research: The results of studies conducted to date in man parallel those of Goldstein et al (Am. J. Physiol. 173:207, 1953) in the eviscerated-nephrectomized dog, and suggest that insulin affects the distribution of D-xylose and L-arabinose, but not of D-arabinose, in body fluids.

Proposed Course of Project: Studies are to be continued with various hexoses and pentoses in an effort to correlate insulin responsiveness with stereochemical configuration of sugars. Also the nature of the insulin response, whether an increase in metabolism, renal clearance or distribution in body fluids of the pentose, will be studied. The physiological disposition and metabolic fate of pentoses will be studied with C^{14} labeled D-xylose, D-, and L-arabinose and D-ribose.

Analysis of NIH Program Activities

10. NIAMD-114-c
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. No cooperating units.

14. No parallel research in the Public Health Service.

16. No publications from this project during calendar year 1955.

17. No honors and awards relating to this project during calendar year 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases

2. Clinical Investigations
LABORATORY

3. Clinical Endocrinology
BRANCH

4. Bethesda
LOCATION

5. NIAMD-115-c
SERIAL NUMBER

6. Metabolism of Vitamins in Diabetes
PROJECT TITLE

7. James B. Field, M.D.
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Title of Project: Metabolism of vitamins in diabetes.

Objectives: To study the metabolism of thiamin, riboflavin, pantothenic acid, niacin, and vitamin B_{12} in diabetics as compared to non-diabetics. To determine whether there is any difference in the metabolism of these vitamins by the diabetics. If such differences do exist, can they be correlated with the presence or absence of any of the complications of diabetes.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	8	55
Outpatient:	Number of patients	1	
	Number of visits	2	

Methods Employed: Both normals and diabetics were put on a standard diet with known amounts of thiamin, riboflavin, pantothenic acid, niacin, and B_{12}. After urinary excretion of these vitamins had stabilized, they were given a test dose of these B vitamins and the urinary excretion was measured. In the diabetics, the urinary excretion of these vitamins was studied both when the diabetes was controlled and uncontrolled.

Major Findings: The evidence suggests that diabetics excrete more riboflavin and pantothenic acid than do non-diabetics. Their excretion of N-methylnicotinamide is less than normal. These patterns of urinary excretion seem to be independent of the presence or absence of complications of diabetes.

Significance to NIAMD Research: If it would be possible to demonstrate a correlation between diabetic complications and vitamin deficiencies then it might be more reasonable to use large doses of vitamins in the hopes of preventing such complications.

Proposed Course of Project: To obtain more information on the excretion of these vitamins in normals and diabetics.

Analysis of NIH Program Activities

10. NIAMD-115-c
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. No cooperating units.

14. No parallel research in the Public Health Service.

16. No publications from this project during calendar year 1955.

17. No honors and awards relating to this project during calendar year 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Clinical Investigation, Metabolic Diseases Branch
LABORATORY OR BRANCH

3. _____
SECTION

4. _____ 5. NIAMD-116-c
LOCATION (IF OTHER THAN BETHESDA) SERIAL NO.

6. Total Energy Metabolism; Studies in Health and Disease
PROJECT TITLE

7. G. Donald Whedon
PRINCIPAL INVESTIGATOR(S)

8. Ernest E. Huber, Jr. and Ronald H. Thompson
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: 1) To establish by complete actual measurement a technique of total energy balance which can be applied to various clinical problems and to fundamental physiological problems of energy metabolism not now understood; 2) To study the influence on total energy consumption and balance of various factors, including climate and the endocrine hormones; 3) To investigate the characteristics of energy balance and their influence on the nutritional state of patients in pertinent disease conditions, such as obesity and cancer.

Methods Employed: Indirect human calorimetry by means of expired air analysis in the Respiratory Chamber, metabolic balance determinations, caloric analysis of dietary intake and excreta.

Patient Material: None

Major Findings: During the early part of the past calendar year the Respiratory Chamber was assembled and its air-conditioning apparatus installed. In testing, serious deficiencies in construction were detected particularly with respect to noise level and to degree of tightness needed for metabolic air measurements. Since the intent of the contract precluded such deficiencies, the contracting company undertook the partial rebuilding of the Chamber. Reconstruction was deemed acceptable and the Chamber was turned over to NIH on September 25, 1955. Since that date the installation of analyzers, recorders and treadmill has been begun.

Significance to NIAMD Research: Previous studies of total energy metabolism were concerned with the influence of various diets on normal metabolism and were carried out with chambers in which oxygen consumption could not be measured directly. Other total energy data have been based on measurements of food consumption or on brief measurements of activity metabolism extrapolated to twenty-four hours. The NIAMD Respiratory Chamber presents an unique facility for the exact and complete measurement of all important expired and excreted products in the study of energy metabolism over long periods of time. Study with this technique has not been made previously of the effects on total energy of various physical and endocrine factors or of disease states. An example of a disease in which energy metabolism may be altered, to be approached with the Respiratory Chamber, is obesity or overweight which predisposes many to the development of diabetes, heart disease, hypertension and atherosclerosis.

Proposed Course of Project: Work will be begun this year with normal healthy individuals a) in delineating the patterns of energy expenditure in certain common activities of daily living and b) in establishing the technique of energy balance. When these techniques are well established, clinical studies will follow.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-116-c
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. No cooperating units.

14. No parallel research in U. S. Public Health Service.

Honors, Awards, and Publications Sheet

16. No publications.

17. No honors or awards.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Arthritis and Metabolic Diseases
INSTITUTE

2. Clinical Investigation, Metabolic Diseases Branch
LABORATORY OR BRANCH

3. _____
SECTION

4. _____ 5. NIAMD-117-c
LOCATION (IF OTHER THAN BETHESDA) SERIAL NO.

6. Studies of the Effects of Various Hormones and of Diet on Calcium and Phosphorus Metabolism in Man
PROJECT TITLE

7. G. Donald Whedon, H. H. Hiatt and David D. Thompson
PRINCIPAL INVESTIGATOR(S)

8. Robert P. Heaney
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: 1. To elucidate the effects of the parathyroid hormone on calcium and phosphorus metabolism in normal subjects and in patients with disturbances in mineral metabolism, and to study the factors regulating the activity of the parathyroid gland in normal and diseased individuals.

2. To investigate the practical aspects of therapy with diet and sex steroid hormones in patients with demineralizing bone disease; the project involves study of the effectiveness of oral steroid preparations for mineral storage, of their effective dosage levels and of the relationship of the latter to the development of undesirable side effects.

NIAMD-117-c
SERIAL NO.

Methods Employed: 1) Renal clearances of phosphorus before and after the administration of parathyroid hormone and under the influence of several dietary levels of calcium and phosphorus. 2) Metabolic balance techniques for study of the relationship of dietary calcium to phosphorus storage and of dietary phosphorus to calcium storage in normal subjects and in patients with altered parathyroid function. 3) Metabolic balance determinations on various levels of calcium and phosphorus intake and before, during and following the administration of sex steroid hormones in patients with demineralizing bone disease.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	8	62
	Adult females	8	84
	Children male	0	
	Children female	1	6
Outpatient:	Number of patients	6	
	Number of visits	15	

Major Findings: 1. Renal clearance studies have resolved the conflict over the mechanism of action of parathormone in increasing urinary phosphorus excretion. By chronic administration of parathyroid extract it has been possible to demonstrate in both normal subjects and hypoparathyroid patients a definite decrease in reabsorption of phosphorus by renal tubular cells.

2. Study of the effect of variations in the dietary intake of calcium and phosphorus on the renal excretion of phosphorus suggest that the level of phosphorus intake plays an important role and that its effect is probably mediated through its influence on parathyroid secretion through which the latter is increased. The intake of calcium also affects phosphate excretion but the means by which this is effected is not clear.

3. In the studies to date on the effects of the gonadal steroids in patients with osteoporosis, oral androgen and estrogen have been shown to be definitely though modestly effective in promoting the storage of bone salts (Ca and P) and protein. Data is being accumulated on the occurrence of side effects of combined therapy in males and females and on the capacity of these orally administered agents to continue their effectiveness over many months of therapy.

Significance to NIAMD Research: 1. The diagnosis of disease of the parathyroid glands is hindered by the absence of specific tests of parathyroid function. This is particularly important in patients who have bone disease of obscure etiology and in those who repeatedly form renal calculi. The diagnosis and removal of parathyroid tumors in many such patients results in permanent cure. It is hoped that information obtained in the present studies may lead to more specific methods of diagnosis in such patients, possibly by measurement of the renal clearance of phosphate under calcium or phosphate loads.

2. Demineralization of the skeleton is a common accompaniment of many incapacitating chronic diseases at all ages, including severe rheumatoid arthritis. Although the value of the steroid hormones administered parenterally has been demonstrated, therapy by this route has usually been abandoned after a brief trial due to inconvenience. If a practical regimen for the necessarily long period (months) of hormone administration in treatment of these bone disorders can be developed from the demonstration of effectiveness of the oral route, a valuable aid to the chronic care of many patients will be provided.

Project Description Sheet Continued

Proposed Course of Project: 1. Studies related to the function and effects of the parathyroid hormone are being suspended because of the loss of key personnel. Numerous data on the influence of varying dietary levels of calcium and phosphorus on the storage of these elements in normal subjects remain to be evaluated. These latter studies will be continued in patients with bone disease.

2. The studies of the effectiveness of gonadal steroid hormones in promoting mineral storage will be continued with metabolic balance determinations in several additional patients. More information is needed on the effective dosage levels of oral steroids, on the relationship of various ratios of androgen-to-estrogen administration to the production of undesirable side effects, and on the capacity of these agents to continue their effectiveness over many months of therapy.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIAMD-117-c
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. No cooperating units.

14. No parallel research in U. S. Public Health Service.

Honors, Awards, and Publications Sheet

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Whedon, G. Donald. Steroid Hormones in Osteoporosis. Proceedings of the Conference on Steroid Hormones and the Aging Process, sponsored by the G. D. Searle Co., May 30-31, 1955, Arden House. In press.

17. No honors or awards.

Analysis of NIH Program Activities

Project Description Sheet

1. Clinical Center
INSTITUTE OR OTHER NIH UNIT

2. Admissions and Followup Department
LABORATORY, BRANCH OR DEPARTMENT

3. Not Applicable
SECTION

4. .
LOCATION (IF OTHER THAN BETH.)

5. 12117
SERIAL NO.

6. Organization and Administration of Admissions and Followup Department
PROJECT OR ACTIVITY TITLE

7. Mr. David F. Burgoon
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. None
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Objectives:

The organization and administration of services for the admission of inpatients and followup patients to the Clinical Center and the operation of scheduled clinic facilities for followup patients. Provide transportation for inpatients and followup patients by government vehicle and taxicab to and from home, railroad station and airport. Photofluorograms are taken on all new patients when their medical condition permits such procedure.

Methods Employed:

New inpatients, readmissions and first visit of followup patients are all processed in an admitting area of the department. The followup patients are scheduled by receptionists in three different wings of the department which have been assigned to specific institutes on designated days of the week. The transportation unit schedules and provides transportation for patients.

A statistical report is attached to indicate the volume of work done in the various activities.

The administration of the department was separated from the Medical Record Department in August. New personnel were appointed to be in charge of the respective departments, each responsible to the Assistant Director of the Clinical Center. Admission functions which formerly were the responsibility of the Medical Record Department are now assigned to the Admissions and Followup department and the Medical Record department confines itself to

handling of the patient's record. Nine employees handle the administrative and transportation responsibilities and ten nursing department personnel are assigned to the followup clinic activities.

The Normal Control Program was also assigned to the Admissions and Followup Department in August. The program has grown considerably in the last year as indicated by the statistics presented below:

	1954	1955
Men	26	36
Women	5	27
Total days served	2,802	6,243

In spite of the increase over last year, the demand for normal control activities is still not being met. Some projects cannot be completed or new ones started because of the limited supply.

Major Problems Encountered:

1. Rapidly growing followup patient load causing increased demand on physical facilities and personnel.

2. Inadequate facilities for handling admission of patients.

3. Increased demand on transportation facilities and personnel.

4. Shortage of normal control patients.

Important Progress or Improvements Achieved:

The number of photofluorograms taken on new patients has increased from 25% to 69%.

Names of referring physicians are sent to the Information office to assist in maintaining a mailing file to inform physicians who wish current information about research programs in the Clinical Center,

Orientation of new Nursing department personnel on the function of the Admissions and Followup and its relationship with the Nursing units.

Reassignment of personnel and changes made in admitting procedure in order to handle the increased volume of patients.

A new filing system established for patients statistical cards to provide more security and easier accessability.

Proposed Course of Activities During the Next Calendar Year:

1. Recruit additional personnel to handle transportation responsibilities and provide adequate coverage during illness and vacation periods for all activities in the Department.

2. Renovation of admitting area to permit more efficient processing of inpatients and followup patients.

3. Revise present clinic schedule to meet the increase patient load and demand on physical facilities in clinic areas.

4. Continue to appraise administrative procedures to provide more efficient methods in all operations of the Department.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12117
SERIAL NO.

11. BUDGET DATA:

12. BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION []

REVIEW & APPROVAL [] TECHNICAL ASSISTANCE []

13. IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

Not Applicable

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH)

Not Applicable

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. 12117
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Not Applicable

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

ANNUAL STATISTICAL REPORT

ADMISSIONS AND FOLLOWUP DEPARTMENT

1955

	1954 January--December	1955 January--December
Number beds activated January 1	244	373
Number beds activated December 31	332	396
Admissions		
Number of inpatient admissions	1452	1889
Number of followup patient admissions	510 (Aug-Dec)	1276
Walkins	no record	98
Emergency	no record	13
Followup		
Total Patients	2455	1292 (Jan-June)
Total number of visits	9371	14,606
Transportation-Number of patients handled		
Government vehicle	no record	715 (Feb-Dec)
Taxi	service not provided	1268 (June-Dec)
Photofluorograms		
All new patients		1644 (April-Dec)
Taken		832 (April-Dec)
Percentage		51

Analysis of NIH Program Activities

Project Description Sheet

1. Clinical Center
INSTITUTE OR OTHER NIH UNIT

2. Professional Services Departments
LABORATORY, BRANCH OR DEPARTMENT

3. Anesthesiology Department
SECTION

4.
LOCATION (IF OTHER THAN BETH.)

5. 12122
SERIAL NO.

6. Anesthesiology Department
PROJECT OR ACTIVITY TITLE

7. C. L. Hebert, M. D.
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. M. G. Bransome (Inhalation Therapy)
J. W. Severinghaus, M. D.; C. A. Nelson, Jr., M. D.; D. R. Dick, M. D.; and
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Project:

Anesthesiology Department, Clinical Center

Objectives:

The primary objective of the Anesthesiology Department has been to provide safe, effective anesthesia and supportive therapy for patients undergoing therapeutic and diagnostic surgical procedures carried out by the surgical services of the several Institutes. This sphere of activity includes the assignment of anesthesiologists to "standby" duties during certain diagnostic procedures not requiring anesthesia, but where circulatory and respiratory depressions of an emergency nature are anticipated. Our second main objective has been to provide for the utilization of all types of inhalation therapy in the care of Clinical Center patients by making available the services of a trained inhalation therapist, together with appropriate apparatus and medical gases.

Research objectives have been necessarily of a secondary nature because of the nature and types of surgical operations performed at the Clinical Center. We have explored several avenues of utilizing new drugs and techniques that would enable us to meet the problems presented in anesthetizing patients under the particular circumstances surrounding surgery done in this Institution. We have participated as members of the operating room team in the surgical research procedures of several of the Institutes, particularly NCI, NHI, and NINDB. One member of our staff has devoted at least half of his time to investigation of basic problems in respiratory and circulatory physiology, both in animals and in man under the auspices of NHI.

Methods Employed:

We have employed all generally accepted anesthetic agents and methods known to the specialty of anesthesiology. We have made clinical trials of a new steroid anesthetic agent and a combination of a potent injectable analgesic drug and its antagonist to meet some of the specific problems posed by the involved type of surgery done at the Clinical Center. Members of our staff have attended or participated in meetings and conferences of National anesthesiological and related societies in order that we may be kept informed of the most recent developments. Field trips have been made to some of the country's leading medical centers to obtain firsthand information in the management of patients undergoing surgery for cardiovascular disease as well as other conditions. During the past year increasing use was made of techniques for lowering patients' body temperature and for lowering the arterial blood pressure in order to accomplish certain surgical procedures without detriment to the patient.

Considerable effort has been devoted to the improvement of methods and techniques for obtaining objective evidence of the patients' condition during anesthesia and special types of surgery. Techniques developed in our Anesthesia Research Laboratory have been adapted for operating room use with the object of decreasing the countless hazards which are ever present under such circumstances.

Patient Material:

Patients coming under our care have been referred to us for pre-anesthetic evaluation prior to being scheduled for surgery. Preanesthetic consultations with the patient's attending physician and the responsible surgeon enable us to plan the anesthesiological management to meet the particular situation presented. Patients receiving inhalation therapy have such treatments prescribed by their physician, sometimes after consultation with an anesthesiologist. Our inhalation therapist provided the necessary equipment, gave instruction regarding its use to nursing personnel, and carried out the more complicated techniques himself.

Almost every patient referred to us for anesthesia or inhalation therapy presents a unique clinical problem that can only be handled by competent anesthetists or inhalation therapists who have had experience with the serious forms of disability possessed by most Clinical Center patients.

Numbers and Kinds of Major Services Rendered:

These data are summarized in Tables I and II. 581 major anesthesiological procedures, an increase of 66% over the number for the previous year, were carried out during the year on patients originating from the surgical services of the several Institutes listed in Table I. It is significant to note that the duration of anesthesia and surgery was in excess of two hours in 60% of all of the procedures performed and was in

excess of three hours 39% of the cases. These observations in regard to the duration of the more complex types of surgical procedures performed at the Clinical Center are in marked contrast to the situation prevailing at other hospitals where the usual types of surgical treatment are accomplished without emphasis on research aspects of the disease with which the patient is afflicted.

In addition, certain diagnostic procedures such as catheterization of the heart chambers and injection of opague media into the cerebral arterial circulation, required the presence of an anesthesiologist on a standby basis for 26 patients during a total of 50 hours. The presence of an anesthetist was deemed necessary in order to cope with any emergencies which might arise during these manipulations.

The Recovery Room Area adjacent to the main Operating Suite was utilized for the care of 31 patients during the immediate postanesthesia-postoperative period. More often, however, the patient was merely kept in the operating room during the critical period immediately following surgery under the care of the anesthetist and responsible surgeons. Many of our procedures require most of one operating day for their completion, seldom is another case scheduled in the same room that day, hence there is no incentive to move the patient from the operating room immediately upon the completion of the operation as must be done in the ordinary hospital. It should also be mentioned that the quality of postoperative nursing care given patients on return to the nursing unit has reached the point where it closely approximates the care which might be given in a formal recovery room set-up. These two reasons have influenced our decision to forego the use of the Recovery Room Area in most instances.

Table II summarizes the numbers and types of inhalation therapy received by patients under the several Institutes. During the past year our inhalation therapist has been called upon repeatedly to devise ways and means for meeting problems which have arisen in connection with the administration of oxygen, carbon dioxide, helium, nebulized medications, high humidity atmospheres, and physical means of assisting respiration. (Intermittent positive pressure breathing, artificial or assisted respiration by means of "iron lung" type respirators, rocking beds, and other apparatus.) The demands made upon our single inhalation therapist have severely taxed his resourcefulness and ingenuity and have necessitated a considerable amount of work after his regular duty hours and during holidays and weekends. As during the previous year, considerable emphasis was placed upon teaching of inhalation therapy techniques to newly employed patient care personnel. During regular scheduled weekly lectures and demonstration sessions, 274 new employees engaged in patient care received this type of instruction. (Footnote Table II).

Major Problems Encountered:

During the past year we have been continually faced with the problem of providing anesthesia personnel in sufficient numbers to carry out the

operative schedules as they are planned by the several surgical services. It has become impossible, because of governmental pay scales, to attract anesthesiologists to our staff to meet the increasing surgical load developing from the expansion of the programs of the several Institutes. We are only able to offer prospective staff members a rate of compensation equivalent to 50% of the yearly income guaranteed by private groups of anesthesiologists to physicians who have completed the minimum of two years training in anesthesiology. Unlike other government hospitals who are faced with the same problem, we are not able to entirely substitute nurse anesthetists for physician anesthetists because of the magnitude of anesthesia and surgical procedures undertaken at the Clinical Center. It has been possible to obtain competent clinical anesthesiologists at governmental pay rates only when the applicant is desirous of obtaining credit for military service or when he has interests along the clinical research lines that might be filled by the unique clinical investigation facilities of NIH. The latter means of attracting anesthesiologists has been emphasized in interviewing prospective staff members. Through the cooperation of NHI one of our staff has been influenced to remain here after completion of his obligatory period of service. A similar cooperative arrangement is being worked out for the coming year with NINDB.

A similar problem exists in regard to employment of inhalation therapists. Civil service regulations preclude the rating of these employees higher than GS-5 (GS-3 for trainees). These grades are not sufficiently high to attract or retain competent therapists who have become proficient through experience and knowledge gained on the job. There are no recognized courses for training such personnel and in the case of the Clinical Center, our techniques and requirements are such that therapists trained in regular hospitals would have to undergo a period of orientation to our methods.

Through careful selection of well qualified nurse anesthetist applicants, we have had the services of a competent nurse anesthetist throughout most of the year. However, the scale of remuneration which we offer is distinctly below that on the outside so that we are at a decided disadvantage in competing for the more desirable personnel. As more physicians can be recruited we plan to add more nurse anesthetists in order to increase the anesthesia coverage for the unusually prolonged procedures.

For the last several months our serious staff shortage has been met only through the employment of a number of outside consultants who arrange their schedules in such a way that they may spend one full day or more with us each week. The anesthesiological procedures carried out at the Clinical Center differ greatly from those in other hospitals. This makes the use of consultants very difficult until they have worked here for a considerable period. Our shortage of anesthesiologists is further compounded by the need for the presence of more than one anesthetist during many of our procedures where it is physically impossible to expect a single anesthetist to cope with all the details of the anesthesia and related procedures for which we are responsible, without jeopardizing the life and the health of the patient. Nurse anesthetists may be used to good advantage to assist in such operations.

Important Progress or Improvements Achieved:

During the past year we have fulfilled the demands placed upon us by the three major surgical services of the Clinical Center. After studying methods used elsewhere, we have devised what we feel to be safe procedures for artificially lowering patients' body temperature. Methods for conduct of anesthesia over long periods have been investigated in order to improve the patients' chances for a successful outcome. Better means for making objective observations of the patients' condition have been devised. Some noteworthy contributions to medical knowledge and literature have been made by the staff member who carried out the research projects mentioned later in this report.

Significance of Activities to Clinical Research Program:

It is obvious that the anesthesiological care for patients undergoing surgical procedures is an integral part of any research program such as those carried out by NCI, NHI, and NINDB. In some instances clinical observations have been carried out on our patients during periods of anesthesia and surgery by our staff member whose work is referred to under Item 13 of this report. These observations have been secondary to the primary purpose for accomplishing surgery for the condition which has brought the patient to this Institution. Searching for ways and means to provide safe and effective anesthesia for patients undergoing prolonged surgery during which the presence of electrosurgical devices precludes the employment of explosive anesthetic gases and vapors, we have explored other avenues which might permit us to meet this problem. A new steroid anesthesia preparation was given a clinical trial on the assumption that it might provide analgesia as well as hypnosis and thus be an improvement over the commonly used intravenous barbiturate anesthetics. Technical difficulties in preparing this drug in a solution adaptable for intravenous use have held this work in abeyance. Seeking to potentiate the effect of light, general anesthesia produced by intravenous Pentothal and nitrous oxide by inhalation, we have investigated the use of a short acting analgesic drug, alphaprodine. When it was discovered that profound respiratory depression resulted from the use of this combination, an additional drug, levallorphan, a physiological antagonist for alphaprodine, was also given with overall satisfactory results in a series of 50 patients.

Proposed Course of Activities During Next Calendar Year:

It is our intention and hope to continue to provide anesthesiological care and inhalation therapy services on a par with the high standards of medical care existing at the Clinical Center. Emphasis will be placed on the recruitment of additional qualified personnel. Clinical research activities in collaboration with the several Institutes will be continued as a means of contributing to the improvement of our methods of patient care and the overall purpose of the Clinical Center.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12122
SERIAL NO.

11.
BUDGET DATA:

12.
BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION []

REVIEW & APPROVAL [] TECHNICAL ASSISTANCE []

13.
IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

Dr. John W. Severinghaus, Anesthesia Department staff member, was assigned to the Laboratory of Chemical Pharmacology of the NHI for the purpose of conducting research related to anesthesiology. While over half of Dr. Severinghaus's time was devoted to this research activity, his services as a clinical anesthesiologist were available whenever he was needed. Other members of Dr. Severinghaus' group as well as the facilities and operating funds for this project were provided by NHI. Research activities of the Anesthesia Research Laboratory were as follows:

1. Respiratory physiology during hypothermia and anesthesia.
2. Distribution of coronary blood flow to the myocardium during hypothermia.
3. Studies on blood pH, pK' and pCO_2 vs. temperature.

14.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

A separate report on the activities listed above has been submitted.

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. 12122
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

The following publications originated from the work done by Dr. Severinghaus and his group:

"Alveolar Dead Space as an Index of the Distribution of Pulmonary Flow" J. W. Severinghaus, M. Stupfel and A. F. Bradley, approved for publication.

"Respiratory dead space increase following atropine in man, and atropine, vagal or ganglionic blockade and hypothermia in dogs." J. W. Severinghaus and M. Stupfel, J. Appl. Physiol. July 1955.

"Respiratory physiology and general anesthesia" R. D. Dripps and J. W. Severinghaus, Physiol. Rev. October 1955.

"The accuracy of blood pH and pCO_2 determinations" J. W. Severinghaus, M. Stupfel, and A. F. Bradley

"Variations of serum pK' with pH and temperature" J. W. Severinghaus, M. Stupfel and A. F. Bradley

"Effect of temperature on pCO_2 and pO_2 of blood in vitro" A. F. Bradley, J. W. Severinghaus and M. Stupfel.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Not Applicable

ANESTHESIOLOGY DEPARTMENT
STATISTICAL REPORT
PART I - ANESTHESIOLOGY
MONTHS OF JANUARY 1955 - DECEMBER 1955
* Administration of Anesthesia and Supportive Therapy for Surgical Procedures.

	Anesthesia Time --- Hours												Total No. of Operations
INSTITUTES	-1/2	-1	-1 1/2	-2	-3	-4	-5	-6	-7	-8	-9	9+	
CANCER	6	24	30	16	46	20	32	23	10	5	1	1	214
MENTAL	3	1	2		1								7
HEART	5	16	20	15	43	25	12	6	8	2	2		154
DENTAL			1										1
ARTHRITIS		2		1	4	6	3	1					17
MICRO.	3	23	11	2	6	4	1						50
NEURO.	1	19	16	18	20	11	7	15	9	9	9	4	138
TOTAL	18	85	80	52	120	66	55	45	27	16	12	5	581

*Anesthesia Time covers only the period beginning with actual induction of anesthesia and ending with the completion of surgery and discontinuance of administration of anesthesia. Time devoted to preanesthetic evaluation of patient, preparation of anesthetic apparatus and materials and immediate postanesthesia managemtn of patient (in operating room, recovery room or patient's room) is not encompassed by these figures.

Of the total number of anesthesiological procedures (581)
346, or 60%, were of more than two hours duration
226, or 39%, were of more than three hours duration
118, or 20%, were carried out for diagnostic procedures done outside main Operating Suite

Anesthesia for surgery on 17 animals (chimpanzees) was carried out at request of NINDB (66 hours)

ANESTHESIOLOGY DEPARTMENT
STATISTICAL REPORT

PART II - INHALATION THERAPY

MONTHS OF JANUARY 1955 - DECEMBER 1955

	*Oxygen tent Therapy		Oxygen by Nasal Catheter or Mask		Administration of Gas Mixtures				**Aerosol Therapy				Consul-tation Service	Resp. & Rock. Bed		Equipment Maintained on Standby Status	
					Continuous		Intermittent		Continuous		Intermittent						
INSTITUTES	Pts	Hours	Pts	Hours	Pts	Hours	Pts	Treatments	Pts	Hours	Pts	Treatments	Cases	Pts	da.	Pt.	Hours
CANCER	34	2406	16	1036	1	120	4	119	1	54	21	1186	7	1	1		
MENTAL	1	1															
HEART	36	3586	39	1137			4	229			19	2096	4	2	31	1	1
DENTAL																	
ARTHRITIS	7	311	4	20			1	44					1				
MICRO.	21	4025	3	447							13	1512	1	3	242	1	2
NEURO.	3	77	2	912							1	6	6	1	19	1	3
TOTAL	102	10406	64	3552	1	120	9	392	1	54	54	4800	19	7	293	3	6

*Includes use of high humidity atmospheres administered by means of oxygen tents.
**Includes use of intermittent positive pressure breathing therapy in conjunction with aerosols in 1232 treatments.
274 new employees received inhalation therapy instructions averaging 1½ hours each:

158 professional nurses
39 practical nurses
59 attendants
15 unit clerks
1 gymnast
2 social service workers

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Clinical Center — INSTITUTE OR OTHER NIH UNIT
2. Professional Services Department — LABORATORY, BRANCH OR DEPARTMENT
3. Clinical Pathology — SECTION
4. — LOCATION (IF OTHER THAN BETH.)
5. 12126 — SERIAL NO.
6. Clinical Pathology Department — PROJECT OR ACTIVITY TITLE
7. George Z. Williams, M.D., Chief — PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY
8. George Brecher, M.D.; Elizabeth Frame, Ph.D.; Arthur Ness, Ph.D. Edna Schnaper, Ph.D.; Andrew Peacock, Ph.D. (NCI, part time); Rose Lieberman, M.S. (NIAID, part time) Hiroshi Nishi, M.S. Residents: Katherine Herrold, M.D., Thomas Dutcher, M.D. — OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Objectives:

The Clinical Pathology Department, Clinical Center, is organized to provide consultation and laboratory services of research precision for patient care in the areas of hematology, clinical chemistry, microbiology, and special diagnostic procedures. In addition, this department provides such laboratory procedures as it is equipped to perform for research pruposes on these patients whenever such provision by centralized facilities and trained personnel is economical of effort and cost.

Methods:

The standard laboratory test procedures have been modified to increase precision and reproducibility and there are included control and check practices to avoid error. A special control laboratory for chemical procedures has improved and completed studies on serum iron procedures, iron binding capacity of serum proteins, and calcium determination by flame photometry. Studies have been continued on the dye binding methods for determination of serum proteins.

Services:

The appended organization chart depicts the laboratory divisions and functions of the department and the assigned personnel. During the calendar year of 1955, 176,310 laboratory test procedures were performed and reported on an average patient census of 267 in the 6 Institutes

for a total of 68,692 patient-work-days. The accompanying chart and table depict the rate of increase as well as the absolute levels of test procedures performed and relates them to the average patient census for each month of the calendar year and the number of patient-work-days - followup visits. An average of 2.2 tests per patient-work-day and followup visit was accomplished and increased progressively during the year to 2.6 in November. It is particularly notable that the increase of test procedures was greater than the concommitant rise in patient census. Compared to 1954, the average patient census increased 50% and the patient-workday-visit 57%, but the test procedure volume rose by 93%.

The major problems during the present year were due to disproportionate workload in relation to the available technical staff. Recruiting of technologists is still difficult because of the short supply of well-qualified persons in this field, the marked disparity in salary scale for technologists in medical laboratories regardless of their training and background in comparison to positions they can obtain in pure research laboratories, there often being a discrepancy of two grade levels. Furthermore, the registers are frequently blocked by incompetent federal employees who cannot do the level of research procedures required in this laboratory, but prevent employment of better qualified persons. Consequently, the activation of new beds and progressive increase in test procedures have consistently overloaded the technical staff. The optimum staffing is represented by an average of 15 test procedures per day per technologist. During 1955, the load fluctuated between 21 and 27, averaging 24.3 for the year. Under these circumstances there was insufficient time for institution of new procedures requested. Advanced instruction of technologists was not feasible, although desirable. Often, peak loads such as the day after holidays, Mondays and Fridays, or during periods when several technologists were sick or on annual leave, the day's work could not be completed in spite of utilizing considerable overtime. The position of the Chief of the Microbiology Service was filled in October by Dr. Edna Schnaper, who is a well-qualified medical bacteriologist.

Progress:

During the past calendar year, further improvements, streamlining and shortening test procedures were accomplished, chiefly through the efforts of the Chemical Control Laboratory under Dr. Ness and outlined under "Methods" above. The organization of the individual laboratories was improved to increase efficiency in time and effort of the technologists. Examination of all specimens is accomplished by grouping them into large batches for each analytical procedure. Efforts have been continued to orient the Clinical Center professional staff to submit all specimens before 9:00 a.m. daily in order to make possible completion of the tests and transmission of reports back to the nursing

units before 5:30 p.m. The investigation of electronic methods for counting red blood cells has been completed, the instrument evaluated and found completely satisfactory. It has been used during the past 8 months for all the red blood counts, completing them at about 5 times the speed and 10 times the accuracy of the best trained technologist. Studies are now being done to apply the electronic counting method to white blood cells.

Significance of the Activities of the Clinical Pathology Department to the Clinical Research Program:

The diagnostic laboratory procedures and followup evaluation of patients by chemical, hematological, and microbiological procedures is basic to all clinical research. It is to be expected that the proper care of research patients will require much more than customary laboratory examinations. The division of so-called "patient care procedures" and "research procedures" is impossible in daily practice in a research hospital, and would necessarily be arbitrary if any such categorical division were utilized in selecting the work to be done in the Clinical Pathology laboratory. Furthermore, when a patient is under treatment by a new procedure, it is to the best interests of the patient and mandatory for his proper care that repeated and pertinent laboratory tests be done to avoid unexpected or hazardous situations. The laboratory data is, nevertheless, of much importance in proper evaluation of the clinical investigation. Analysis reveals that the amount of clinical pathology required depends considerably on the type of clinical research being done as tabulated below:

Average Montly Test-Procedure Volume by Patient Categories

Cancer	86
Metabolic	56
Heart	53
Microbiological	112
Neurological	20
Mental	5

Examples of Progress and Significance of Special Procedures and Services Required by the Research Nature of the Patients in the Clinical Center

1. Adaptation of Research Technics. The ultraviolet television microscope has been developed and applied to examination of fresh living bone marrow preparations of patients for rapid and more accurate differential diagnosis of unusual anemias and leukemia.

The recently publicized research on transanimase in the blood of vicitms of coronary thrombosis has been adopted and applied in our laboratories for use in all suspected cases in the Clinical Center.

2. Research development of new procedures. An electronic counting instrument, developed by an engineer, has been adapted, modified and evaluated for counting red blood cells. With this instrument, counts can be done with high accuracy and 5 time faster than by the best trained technologist using the customary microscopic method. Its use makes possible the determination of population size distribution of red cells in a patient never before feasible. This technic has uncovered a new borderline type of anemia.

 Research in methodology for accurate measurement of iron in blood serum yielded a superior technic. This has been applied to patients and several cases of unsuspected deficiency of iron were discovered. This procedure now constitutes an important addition to diagnostic procedures.

3. Improvement of Accuracy and Information. A very important innovation in diagnostic tests is serial repetition of more accurate procedures to follow and control life saving treatment of gravely ill patients. Serial determinations of blood acidity or alkalinity and carbon dioxide content are done every one, two or four hours to closely and accurately guide therapy.

 Serial daily blood platelet counts are done to control chemotherapy of leukemia.

 Serial bacteriological antibiotic sensitivity tests are done to control antibiotic treatment of patients with serious infections.

 These serial procedures must be more accurate than those customarily performed, and are usually not feasible in the routine laboratory.

Residency Training Program:

An approved residency training program in clinical pathology was initiated on July 1, 1955, at which time two residents, Dr. Dutcher and Dr. Herrold, were appointed from the Public Health Service. The residents, are rotated for a 3 month period in each of the clinical pathology services, hematology, clinical chemistry, microbiology, and for the fourth period of the first year, in administrative and organization training under the supervision of the chief of the department. The second year of training, the residents will elect one of these areas for full time and continue intensive and advanced work in this area under the direct supervision of the chief of the laboratory, and will take considerable responsibility for the supervision of the work done in that area.

Proposed Activities for the Next Calendar Year:

The objectives of this department are such that our services will be required as long as the Clinical Center operates for clinical reasearch. In addition, specific objectives include management analysis studies to improve intradepartmental communications in order to save time, space organization for more efficiency in assignment of personnel to tasks, and the improvement of record systems. It appears that the space in this department is now occupied at optimum capacity, but the patient census is only about two-thirds of that anticipated on full bed activation by fiscal year 1958. Therefore, it is mandatory that reorganization and space assignment in a more efficient manner be made in order to accomodate a larger staff and an estimed increase in volume of 100% in laboratory test procedures over the present level. The followup patient laboratory will be open during the next fiscal year and will take some of the volume from the present space. Recruiting will be further emphasized in order to fill present vacancies and those required for increase in the test procedure volume. An effort will be made to provide further variation in test procedures which are frequently requested by the patient care staff. A program of advanced training of the technologists in the special areas of hematology, chemistry, microbiology, and virology is being instituted. We will emphasize mechanization, electronic and mechanical methods for substituting more arduous, time-consuming manual procedures in as many methods as possible. A diagnostic virology laboratory will be opened in July with the assignment of a full-time virologist. The resident training program will be expanded to include three first year clinical pathology residents and two or three second year clinical pathology residents. This program is integrated with that of the Pathologic Anatomy Department to provide a complete pathology training of four years.

R.P.C. - 2
December 1955 Analysis of NIH Program Activities

Budget Data Sheet

10. 12126
SERIAL NO.

11.
BUDGET DATA:

12.
BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION []

REVIEW & APPROVAL [] TECHNICAL ASSISTANCE []

13.
IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

NCI (research project of Chief - George Z. Williams, Serial No. 201)

14.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH)

Not applicable

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. 12126
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Not applicable

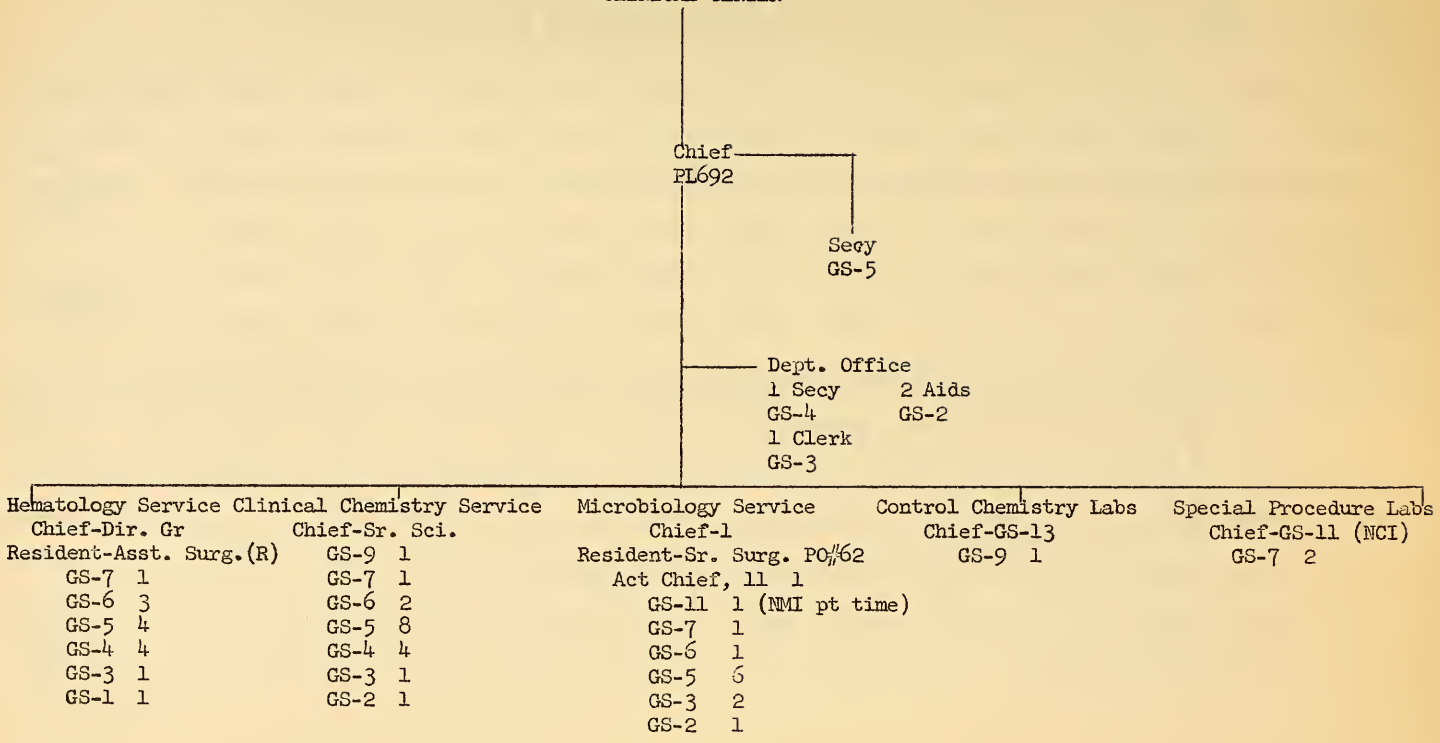
17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Not applicable

ORGANIZATION CHART

DEPARTMENT OF CLINICAL PATHOLOGY

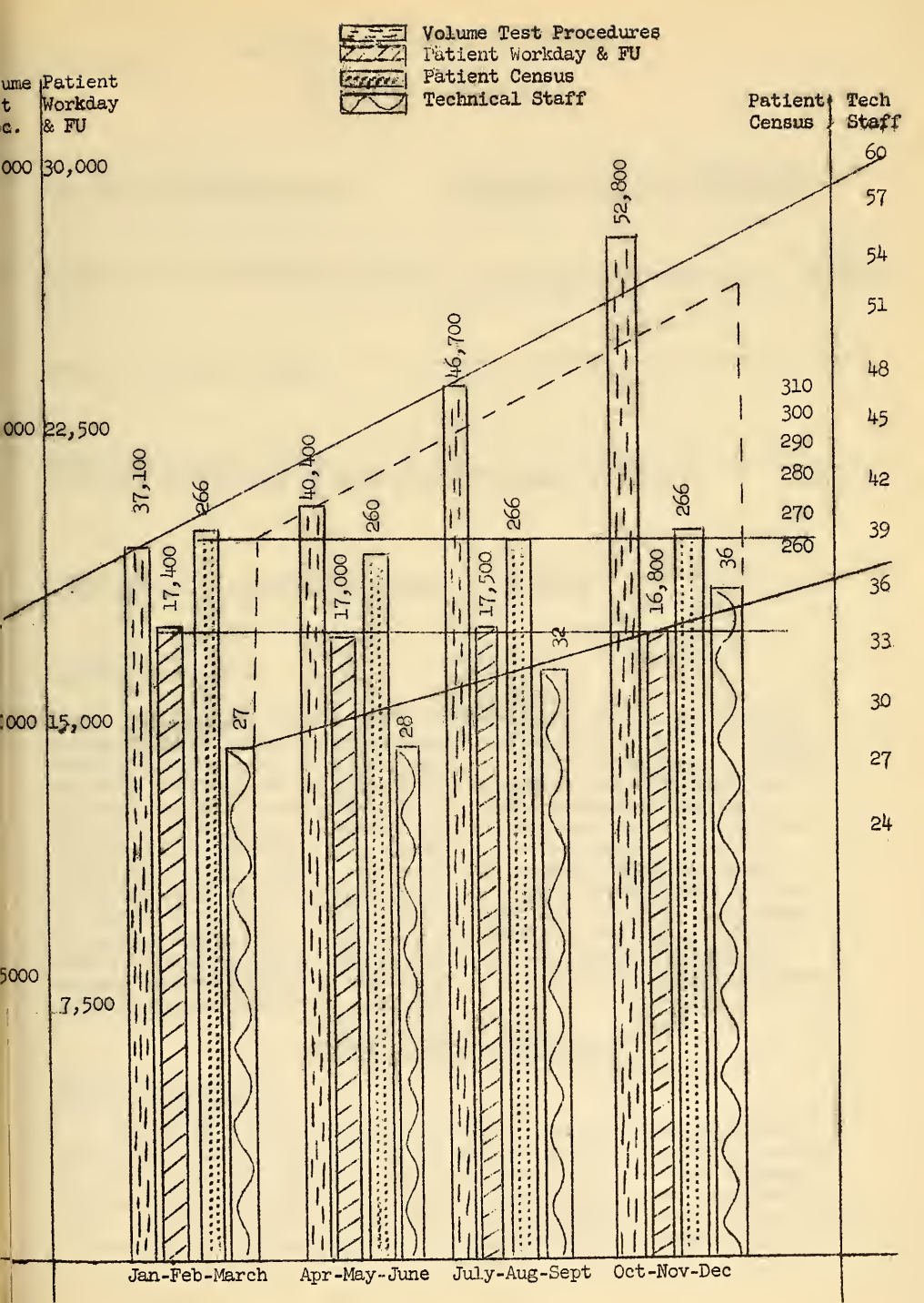
CLINICAL CENTER



1955

	Jan.	Feb.	March	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total or Av.	Increase No.	%
Av. Pt. Census	264	279	261	278	263	269	274	257	266	273	278	251	267	89	50
Pt. Workday	5544	5301	6417	5590	5510	5917	5691	6026	5874	5733	5560	5529	68,692	24,944	57
Test procedures	12,156	11,559	13,412	12,687	13,123	14,566	13,860	16,532	16,369	17,207	18,771	16,489	176,310	84,650	93
Test/Pt. Wkd.+ FU	2.0	2.0	2.1	1.9	2.0	2.2	2.2	2.2	2.3	2.4	2.6	2.5	2.2	-	-
Test/Patient	43	36	48	46	47	50	52	67	70	68	69	63	54	+12	28
Technical Staff	24	29	29	30	27	28	31	32	32	34	38	36	30	+14	88
Test/Tech./Day	25.7	23.0	21.5	22.8	25.5	24.5	24.6	24.0	27.0	26	26.9	22.0	24.4	+0.8	3
NCI	65	60	73	64	71	79	80	118	105	108	109	100	86		
NMI	65	57	63	75	73	79	114	169	196	170	143	141	112		
NHI	47	41	58	49	51	59	43	47	48	60	67	63	53		
NIAMD	52	52	63	59	63	63	54	47	50	51	52	70	56		
NINDB	21	21	23	25	24	21	18	17	21	16	18	21	20		
NIMH	9	5	7	3	4	4	3	5	3	3	6	6	5		

LEGEND: Chart indicates the relation of average patient census to volume test procedures for the calendar year 1955. Patient census and the average patient workdays per quarter remained constant. The test load increased markedly, and although the average technical staff increased somewhat, it was insufficient to provide for maximum accuracy.


Volume Test Procedures
Patient Workday & FU
Patient Census
Technical Staff
Patient Workday & FU
Patient Census
Tech Staff
30,000
22,500
15,000
7,500
60
57
54
51
48
45
42
39
36
33
30
27
24
310
300
290
280
270
260
37,100
17,400
266
27
40,400
17,000
260
28
46,700
17,500
266
32
52,800
16,800
266
36
Jan-Feb-March
Apr-May-June
July-Aug-Sept
Oct-Nov-Dec



R.P.C. . .
December 19551955

Analysis of NIH Program Activities

Project Description Sheet

1. The Clinical Center — 2. Professional Services Departments
INSTITUTE OR OTHER NIH UNIT — LABORATORY, BRANCH OR DEPARTMENT

3. Clinical and Professional Education — 4. _____ — 5. 12112
SECTION — LOCATION(IF OTHER THAN BETH.) — SERIAL NO.

6. _____
PROJECT OR ACTIVITY TITLE

7. Chief, Dr. Murray C. Brown
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. _____
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

The Clinical and Professional Education Branch of the Clinical Center is concerned with formal educational activities for professional people, the conduct of staff meetings and other professional activities relating to the growth of professional competence and the maintenance of standards of clinical care. As an interim responsibility this office has also been responsible for Volunteer Services in the Clinical Center and the planning of communication services having potential educational implications. Its Chief also maintains a relationship to the Director of NIH and organizes and conducts educational programs of overall importance to the National Institutes of Health. Its Chief represents the National Institutes of Health in a variety of Public Health Service educational activities. During 1955 this office has assumed responsibility for directing the in-service training activities of the National Institutes of Health.

CLINICAL CENTER ACTIVITIES

Clinical Fellowships

There are now six programs for the training of clinical fellows which have received approval for one or more years of Residency training from the appropriate American Specialty Board. Four of these approvals were secured in calendar 1954. Four clinical fellows were appointed in 1955. Clinical associates whose work closely parallels that of the clinical fellows, but who are not involved in formal training programs totaled 56.

Staff Meetings

There have been a total of 14 meetings of the combined clinical staffs of the National Institutes of Health during 1955. Eight of these meetings were combined clinical staff meetings. Three were Clinicopathologic Conferences and the remaining three were Quarterly Staff Meetings devoted to a review of professional service standards. Distinguished professors of medicine were the discussants for the three Clinicopathologic Conferences.

Education Committee of the Medical Board

This Committee met a number of times under the chairmanship of the Chief of this Branch and developed basic policies governing the conduct of staff meetings and the appointment and status of clinical fellows. This Committee, augmented by members of the Public Health Service Committee on Residencies and Internships, also participated in the selection process whereby clinical fellows were appointed.

Volunteer Services

Regularly scheduled Volunteer Services have been limited to those provided by a cooperative arrangement between the American Red Cross and the Clinical Center, whereby the Gray Lady Corps of the Montgomery County Chapter created a Clinical Center Unit for this purpose. On January 1, 1954, the first class of Gray Ladies had been on duty for seven months. By January 1, 1956, three additional classes had been graduated and the group had grown from less than 30 to 77 Gray Ladies, despite the normal attrition to be expected in a volunteer group. During 1955, this group rendered 13,000 hours of volunteer service, as compared with 9,734 hours of service in the previous year.

In addition to these services a number of volunteers participated in activities in the recreational therapy area on a "one time" visit basis.

Television

During 1955 the radio and television distribution system for the Clinical Center moved from a design stage to the contract stage. In April 1955, a series of experiments with television were conducted in the Clinical Center and the results were filmed. With the assistance of the Navy Special Devices Center and the Navy Photographic Center these materials were evaluated and a filmed report was prepared. This report has been reviewed at NIH and before a number of hospital and professional organizations. Complete protocol film on certain experiments has been made available to interested people for appraisal in connection with their personal projects. Several scientific papers based on these studies are in press.

With the assistance of Signal Corps Engineers the essential equipment needed for the completion of the television portion of the distribution system has been specified and awaits authority for it's purchase. A proposal for a two year study of the applications of television through a joint undertaking with Navy has been prepared and awaits approval.

ACTIVITIES CONDUCTED FOR NIH

Orientation of New Employees

The National Institutes of Health has had an organized program for the general orientation of new employees since July 1953. During 1955 seven series of orientation programs were conducted. A total of 1,204 people attended the sessions. Two special orientation sessions were planned and conducted. These were for the top level Public Health Service employees from the four operating Bureaus.

Graduate School

In 1954, a program of formal "out of working hours" courses was organized in cooperation with the Graduate School of the Department of Agriculture. Special committees of NIH scientists and other groups with special talents and interests were created to select the content and guide the conduct of these courses. The courses are offered at the employee's expense at the current rates charged by the Graduate School.

In the spring semester of 1955, there were 180 people enrolled in 17 courses. In the fall semester of 1955, there were 321 people registered for 18 courses.

Inservice Training

In November of 1955 this office secured the services of two training officers whose duties will be to stimulate and coordinate instructional programs designed to improve the work performance at NIH. By December 30, 1955, this staff had completed a base line study of educational programs and problems at the NIH. A program of in-service training will be initiated on the basis of this information.

Miscellaneous

The Chief of this Division has been active on the Interagency Committee on Medical Education for National Defense throughout the year and has assisted in the planning of presentations for Deans and Coordinators of Medical Schools Participating in the Mend Program. He has attended national meetings of those organizations dealing with medical education.

R.P.C. - 1 (Concl'd) Analysis of NIH Program Activities

Project Description Sheet

There has been an increasing demand for programs for medical students in local medical schools regarding the PHS and NIH functions. A number of two hour programs have been set up for these students and for occasional groups visiting from a distance.

Activities of the Residency and Internship Committee of the PHS have made increasing demands on this office and the creation of a new Committee on the Commissioned Reserve has required some time. Planning for a PHS program of summer employment for medical and dental students started in December. In 1956 the educational aspects of this program will be recognized and much of the NIH portion of its administration will evolve in this office.

Counseling of younger staff members regarding their professional development has been increasing. A number of new programs concerned with professional education have been designed and are in the formative stage.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12136
SERIAL NO.

11.
BUDGET DATA:

12.
BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION []

REVIEW & APPROVAL [] TECHNICAL ASSISTANCE []

13.
IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Not Applicable.

14.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not Applicable

R.P.C. - 3

Analysis of NIH Program Activities
Honors, Awards, and Publications Sheet

15. 12112
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

Project Description Sheet

1. Clinical Center — INSTITUTE OR OTHER NIH UNIT
2. Professional Services Department — LABORATORY, BRANCH OR DEPARTMENT
3. Dental Department — SECTION
4. _____ — LOCATION (IF OTHER THAN BETH.)
5. 12125 — SERIAL NO.

6. Dental Department
PROJECT OR ACTIVITY TITLE

7. Dr. Ralph S. Lloyd
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACITIVITY

8. Dr. Peter J. Coccaro, Dr. Herbert Swerdlow
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Project:

Dental Department, Clinical Center

Objectives:

The objective of this Department is to provide a complete diagnostic and dental treatment service for the various institutes of the National Institutes of Health.

Methods Employed:

It was the aim of the dental department to perform a complete dental examination on every patient admitted to the hospital. Recommendations for treatment were made and where requested, dental treatment was rendered to those examined. Full mouth dental radiographs were included in every dental examination.

Number and Kinds of Major Services Rendered:

The Dental Department is prepared to and did render every type of dental treatment which was found necessary to perform on the patients examined. The number of dental treatments are progressively increasing from month to month. When the patients are examined we attempt to find out whether they have a private dentist and we try to evaluate their ability to afford private dentist service. If at all possible we recommend that they return to their private dentist for their work. It is our observation, however, that a great percentage of patients in the Clinical Center are badly in need of dental services and cannot afford the same. The majority of patients examined have dental conditions which directly affect their health and their utilization in research projects in other institutes.

Major Problems Encountered:

We are still trying to obtain another staff officer for routine work in the Dental Department. The man has been selected and will be transferred here as soon as a replacement is obtained for him in his present position.

The Dental Clinic may now be considered mature. It is meeting all the requirements, it functions smoothly and our only problem is additional staffing to take care of increased work load.

Our Growth and Development Section is suffering due to the lack of professional staffing. We have in the past year lost services of two orthodontists who were running a very successful clinical research study in Growth and Development. It is quite possible that we may obtain services of another orthodontist in the near future to continue both the work for which we are responsible on the previous patients and to do further research in Growth and Development.

Important Progress Achieved:

Additional dental units were added to the Dental Department in the past year bringing the physical aspects of the clinic up to near full planning. It is possible to add two more units in the future. Increase in demands due to relative increases by the institutes were successfully met during the year.

Significance to Clinical Research Program:

Many clinical projects of other institutes were facilitated by dental treatment. Rehabilitation of masticatory apparatus in debilitated patients was of help. Emergency dental procedures are constantly arising wherever a large number of patients are assembled and were successfully treated in every case. Many special dental consultations related to patient care were handled. The dental department furnished facilities and in some cases some personnel time for clinical dental research.

Several clinical research projects are being participated in by the staff of the Dental Department. A study on the effect of premature contacts on the periodontium and an evaluation of drilling techniques and their effect on the health of the pulp is nearly completed. The staff of the Dental Department is participating in these projects. A number of other clinical studies by the staff of the National Institute of Dental Research require the facilities of the Dental Department.

In the field of maxillo-facial prostheses, a number of patients of the National Cancer Institute, the National Institute of Neurological Diseases and Blindness and the National Institute of Arthritis and Metabolic Diseases were benefited by special prosthetic devices. Clinical

research in methods of treatment of cleft palate patients utilizing prosthetic devices is under study.

Proposed Course of Project:

It is anticipated that a member of the staff of the Dental Department of the Clinical Center will be sent to post-graduate school in orthodontia and Growth and Development. When his training is completed he will return to our organization.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12125
SERIAL NO.

11.
BUDGET DATA:

12.
BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION []

REVIEW & APPROVAL [] TECHNICAL ASSISTANCE []

13.
IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

The Dental Department has instituted several cooperative research studies in conjunction with the National Institute of Dental Research They are:

(1) The effects of premature contacts and trauma on health of the periodontoclasia.
(2) Tissue resistance.
(3) Stomatitis and gingivitis.
(4) Affects of drilling techniques on the dental pulp.
(5) Cleft palate prosthetics.

14.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH)

Not applicable

R.P.C. - 3 Analysis of NIH Program Activities
December 1955

Honors, Awards, and Publications Sheet

15. 12125
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Clinical Research Activities of the National Institute of Dental Research.
Ralph S. Lloyd, D.D.S.
Published in American Journal of Public Health, Vol. 45, No. 5, May, 1955

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Clinical Center
INSTITUTE OR OTHER NIH UNIT

2. Professional Services Department
LABORATORY, BRANCH OR DEPARTMENT

3. Diagnostic X-ray Department
SECTION

4. _____
LOCATION (IF OTHER THAN BETH.)

5. 12124
SERIAL NO.

6. Diagnostic X-ray Department
PROJECT OR ACTIVITY TITLE

7. Dr. Theodore F. Hilbish
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. Dr. Eugene L. Bronstein, Dr. Byron E. Besse, Jr., Dr. Lee B. Lusted
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Project:

Diagnostic X-ray Department, Clinical Center

Objectives:

The objective of this Department is to provide a complete diagnostic radiological service for the various Institutes of the National Institutes of Health.

Methods Employed:

In order to accomplish this objective, X-ray equipment was procured and trained personnel were employed. All types of radiographic examinations were performed during the calendar year 1955.

Number and Kinds of Major Services Rendered:

A total of 21,958 X-ray examinations, exclusive of animal radiography, were performed during the year 1955. Table I indicates the workload by Institutes on a monthly basis for inpatients and outpatients. The same data are provided on an annual basis in Table II, while Table III depicts the special radiographic studies completed within the Department.

In addition to the conventional and special radiographic procedures portrayed in the above-mentioned tables, a photofluorographic film reading service was provided by the Department for employees of NIH. A photofluorographic survey was conducted during the calendar year 1955 and these films were read and several cases of important pathology were discovered by this manner in asymptomatic personnel.

A method of selective intracardiac angiocardiography was instituted during the calendar year 1955 to provide individual cardiac chamber evaluation for the N.H.I. physicians. In addition to conventional diagnostic procedures, many special radiographic studies were consummated as specified in Table III.

A photofluorographic film screening service was instituted in the Admissions and Followup Department for inpatients and outpatients admitted to the hospital. The maintenance of this photofluorographic unit, film processing, and film reading were provided as a service of the Diagnostic X-ray Department.

Major Problems Encountered:

There were two moderately serious problems encountered of a technical nature. These involved the fogging of X-ray films and the discoloration of fixing solutions. The first problem, i.e., fogging of films, was found to result from a varnish product utilized in the film carrying cylinders. The fogging of films was promptly eliminated following determination of its causative factor. The discoloration of the fixing solution resulted secondary to the silver reclaiming process. This discoloration occurred as a result of an electrolytic action between the silver collecting apparatus and the processing chemicals. This problem likewise was readily solved when the cause was determined.

The problem of procurement of suitably trained personnel persisted during the calendar year 1955 largely because of noncompetitive salaries of the government for radiologists.

Important Progress Achieved:

The radiographic method of serial uretography developed by the Diagnostic X-ray Department was improved and a new contrast substance was utilized in place of the 70% Urokon preparation formerly used for this examination. Selective intracardiac angiocardiography was instituted in the Diagnostic X-ray Department in the calendar year 1955. This is a new and improved method of cardiac chamber evaluation. This method has been used in several foreign countries but its use in the United States has been extremely limited and only in recent months has it been adopted in this country.

Additional radiographic equipment was procured and installed permitting an expanded radiographic service. All requests for conventional and special radiographic procedures were met by the Diagnostic X-ray Department.

Significance to Clinical Research Program:

As in preceding years, most of the clinical research programs were dependent upon X-ray control. The evaluation of the efficacy of many drugs, viruses and antibiotics as well as radiographic therapy was dependent upon periodic X-ray examinations. The research program of NHI was promulgated by the introduction of selective intracardiac angiocardiography. All Institutes were dependent to varying degrees upon radiographic control in their research activities.

Proposed Course of Project:

The needs of the various Institutes of Health from the standpoint of diagnostic X-ray services will be met by the Diagnostic X-ray Department. Toward this end, suitable trained personnel and necessary equipment will be provided to meet the needs as they develop. Equipment to provide all types of radiographic procedures on animals within the Clinical Center has been requisitioned during calendar year 1955 and will be installed early in the succeeding year. The requests for this type of service have reached a sufficient level to justify the procurement of additional facilities for animal radiography in the Clinical Center.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12124
SERIAL NO.

11. BUDGET DATA:

12. BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION []

REVIEW & APPROVAL [] TECHNICAL ASSISTANCE []

13. IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

The method of serial uretography developed by the Diagnostic X-ray Department has been further refined and improved. A 50% Hypaque solution has been found to be more efficacious and less toxic than the 70% Urokon previously utilized for serial uretography.

A project is underway at the present time to develop a more suitable film carrying cylinder for use in the Diagnostic X-ray Department. The Instrument Shop has just completed the construction of two cylinders made of plastic for trial use in the Department. These were constructed according to our instructions and will require additional modification. We have every reason to believe these cylinders will prove to be a great deal more satisfactory than the previous cylinders which were heavy and required frequent servicing. This project is mentioned since we have received numerous inquiries from radiologists and hospitals as to the best method of film transport.

Two cooperative research studies are in progress involving the evaluation of two gallbladder dyes and three pyelographic materials for intravenous pyelography. These products are all furnished by the respective manufacturing concerns without cost to the government. They are being investigated to determine the most suitable agent for cholecystography and intravenous pyelography. A similar study was completed during the fiscal year 1955 involving the evaluation of 50% Urokon, 70% Urokon and 50% Hypaque. Our results clearly indicated that the 50% Hypaque was considerably less toxic to the patient and provided

radiographic studies superior to the 50% Urokon and equal to those obtained with 70% Urokon. As a result, 50% Hypaque is now used routinely for intravenous pyelography in the Diagnostic X-ray Department.

A head-holding device has been constructed for bilateral cerebro-arteriography. This device was devised and modified as experience dictated for use on the Schonander biplane radiographic unit. This head-holder has been constructed of balsam wood. It is inexpensive, very light, and does not cast a shadow of sufficient degree to interfere with the X-ray films obtained with its use. We have also received inquiries concerning the construction of this apparatus.

A cooperative study was undertaken with the Dupont X-ray Corporation and the Radelin Division of the U.S. Radium Corporation relative to evaluation of fluorescent screens used in conjunction with the Schonander biplane radiographic unit. A series of screens were evaluated and the screen of the U.S. Radium Corporation was found to be a great deal more satisfactory than the screen provided by the Schonander Company in Sweden.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH)

Not applicable

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. 12124
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Leone, Nicholas C. Stevenson, Clyde A. Hilbish, T. F. Sosman, Merrill C.	A Roentgenologic Study of a Human Population Exposed to High-Fluoride Domestic Water - A Ten-Year Study. The American Journal of Roentgenology, Radium Therapy, and Nuclear Medicine, Vol. 74, No. 5, 874-885, November 1955.
Hilbish, T. F.	News Release on new diagnostic procedures. Hospital Progress, May 1955.
Hilbish, T. F.	News Release on Serial Uretography, X-ray News (General Electric), Vol. 27, No. 5, May 1955.
Bronstein, E. L.	Angiocardiography Today. Accepted for publication in Cathode Press.
McAfee, J. G. Hilbish, T. F. Stewart, K. R.	Angiocardiography in the Preoperative Diagnosis of Mitral Stenosis and Insufficiency. Accepted for publication on September 9, 1955 in Radiology.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

The Chief of the Diagnostic X-ray Department presented a paper at the Fifth Inter-American Congress of Radiology. He was also a committee member for this meeting. Mr. Morel was elected chairman of the committee on X-ray protection of the American Society of X-ray Technicians. Dr. Bronstein presented a paper "Angiocardiography Today" at the Eighth Annual Conference on Electrical Techniques in Medicine and Biology. The Chief of the Diagnostic X-ray Department was appointed chairman of a symposium on angiocardiography at this same meeting.

TABLE I
NUMBER OF EXAMINATIONS
(January 1955 through December 1955)

MONTH	INSTITUTE (By Type of Patient)																TOTAL
	NCI		NHI		NMI		NIAMD		NIMH		NINDB		NIDR		EHS		
	In-pt.	Out-pt.	In-pt.	Out-pt.	In-pt.	Out-pt.	In-pt.	Out-pt.	In-pt.	Out-pt.	In-pt.	Out-pt.	In-pt.	Out-pt.	In-pt.	Out-pt.	
JAN.	372	43	193	50	153	87	263	23	24	--	131	18	--	3	--	427	1787
FEB.	332	21	168	18	164	67	100	11	4	1	107	56	--	10	--	325	1384
MARCH	380	91	164	18	137	119	126	26	8	5	151	59	--	6	--	389	1679
APRIL	336	60	148	33	124	44	325	35	12	--	180	37	--	10	--	[illegible]	[illegible]
MAY	387	53	227	21	126	43	178	10	19	--	174	15	--	29	--	232	1514
JUNE	546	116	228	35	110	59	195	10	13	--	135	35	--	11	--	524	2017
JULY	560	71	152	35	136	76	83	34	10	2	136	24	--	3	--	373	1695
AUG.	687	123	195	51	105	70	178	67	9	1	115	45	--	23	--	386	2055
SEPT.	551	132	135	34	82	77	170	84	9	--	151	73	--	9	--	404	1911
OCT.	716	186	138	59	167	70	144	42	8	--	141	31	--	13	--	439	2154
NOV.	616	149	145	42	168	67	97	62	7	--	164	50	5	12	--	405	1989
DEC.	532	191	123	27	138	67	117	55	5	--	204	90	10	5	--	532	2096
TOTAL	6015	1236	2016	423	1610	846	1976	459	128	9	1789	533	15	134	--	4769	21958

Diagnostic X-ray Department

TABLE II

NUMBER OF X-RAY EXAMINATIONS

INST.	TYPE OF PATIENTS		TOTAL
	IN	OUT	
NCI	6015	1236	7251
NHI	2016	423	2439
NMI	1610	846	2456
NIAMD	1976	459	2435
NIMH	128	9	137
NINDB	1789	533	2322
NIDR	15	134	149
EHS	--	4769	4769
TOTAL	13549	8409	21958

Diagnostic X-ray Department

SURGICAL PROCEDURES PERFORMED IN DIAGNOSTIC X-RAY DEPARTMENT
1955

MONTH	TYPE OF SPECIAL PROCEDURE							
	Angiocardiograms	Aortograms	Cerebroarteriograms	Bronchograms	Cystoscopy with Retrograde Pyelogram	Myelograms	Pneumoencephalograms	Other Special Surgical Procedures*
JANUARY	7	5	1	1	2	2	16	2
FEBRUARY	10	1	1	--	1	2	26	4
MARCH	3	5	10	1	2	2	30	3
APRIL	10	2	3	--	3	1	33	2
MAY	7	3	1	--	1	--	35	4
JUNE	10	2	7	--	3	--	22	2
JULY	4	4	6	3	5	1	20	4
AUGUST	11	2	6	1	4	--	19	5
SEPTEMBER	4	--	5	2	2	--	22	1
OCTOBER	10	5	3	1	2	1	16	--
NOVEMBER	5	3	1	1	3	1	14	5
DECEMBER	9	5	5	1	1	--	18	3
TOTAL	90	37	49	11	29	10	271	35

*Other Special Surgical Procedures include the following: Pulmonary angiograms; Presacral Air Insufflation; Ventriculograms; Hepatic Vein Catheterizations; Phlebograms; Brachial Arteriograms; Splenograms; Nephrograms; Femoral Aortograms; Sialograms; and Fistulous Tract Injections.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Clinical Center
INSTITUTE OR OTHER NIH UNIT

2. Office of Director, NIH C.C.
LABORATORY, BRANCH OR DEPARTMENT

3. Employee Health Service Branch
SECTION

4. _____ 5.12171
LOCATION(IF OTHER THAN BETH.) SERIAL NO

6. Employee Health
PROJECT OR ACTIVITY TITLE

7. John M. Lynch, M.D., Chief
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. Frances S. Wolford, Head Nurse
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Objectives:

The Employee Health Service is charged with the responsibility of assisting employees to maintain their health at the highest possible level, primarily through the provision of preventive medical services; such as, readily available health guidance, health education, and early case finding opportunities. The Employee Health Service program likewise includes the medical aspects of the control of hazardous working conditions; as well as the prompt and efficient management of work-connected injuries and illnesses that occur in spite of efforts to control hazardous working environments. In addition to the benefits received directly by the employee, the research program benefits in that a higher level of effective production can be expected in employees whose health needs are being met.

Methods Employed:

Every employee visit, from the initial pre-employment physical examination at which time a baseline inventory is obtained, is utilized to provide health guidance and education. When necessary, employees are referred to private medical facilities in the area, as treatment per se, without the knowledge of an individual's personal physician, is not available in the Employee Health Service. Qualified Public Health Nurses provide the bulk of health advice and education, as the Medical Officer is able to interview and examine only the more complicated and involved cases.

Working closely with the Safety and Sanitary Engineering Branches through the Environmental Health Advisory Group, the Employee Health Service provides consultation on the health aspects of environmental hazards. Considerable time is also spent in other administrative and service activities concerned with employee relations and suitability determinations.

Numbers and Kinds of Major Services Rendered:

Total visits	20,959
Visits for occupational injuries and diseases	2,633
Immunizations	2,413
Preemployment physical examinations	1,351
Laboratory examinations	6,475
Referrals to personal physicians (revised stds.)	1,681

Major Problems:

1. Inability of the single Medical Officer to see as many employees who feel they may have a problem which requires his personal attention.

2. Continued, but considerable improved, relative low level of understanding by many administrative and operating personnel of the preventive medical nature of Employee Health Service objectives; and of the limitation of medical care services, as stipulated by existing laws and regulations.

Important Progress of Improvement Achieved:

1. A greater emphasis on preventive health programs resulted in collaborative research projects with several of the Institutes and provided mutual benefits, both to employees and the Institutes. Periodic vaginal cytology examinations, trichomonas control, beta hemolytic streptococci control, dysmenorrhea control, are major examples. Over 3000 employees received periodic chest x-rays, while 1600 were immunized with Influenza Virus Vaccine.

2. The Environmental Health Advisory Group, with representation from the Employee Health Service, Safety and Sanitary Engineering branches, was formed; thereby facilitating the control of environmental health hazards at NIH, many of which are unique and potentially very dangerous. Poliomyelitis and other infectious disease hazards have received much attention during the year. A joint survey of health hazards in Building 6 has initiated a program which will eventually cover all other buildings on the reservation.

3. A new Health Unit in Building 13, which will more conveniently service employees in the southern sector of the reservation is expected to be completed during 1956.

4. The assignment of two medical students to the Employee Health Service during the past summer significantly increased the number of employees which could be seen by a medical officer. The medical students and the Public Health Service's commissioned officer recruitment program likewise benefited.

Proposed Course of Activities During the Next Calendar Year:

1. More employees will be able to receive the personal attention of a medical officer as a result of the creation of an additional position for a physician, which it is hoped will be filled during the summer of 1956. Further efforts to acquaint employees with the Employee Health Service's basic objectives and limitations, as set by law and regulation, may be necessary as a result of the possibility that some employees might assume that two medical officers would now be able to provide a more complete type of Medical care program.

2. With the stabilization of the NIH population at its present level, the added responsibilities placed upon the Employee Health Service during the rapid growth period of NIH will shortly be markedly decreased. At that time, and continuing in the future, it is hoped that overall improvement of present services to employees and to management can be made.

3. With the proposed additional physician, more medical assistance can be given to the Sanitary Engineering and Safety Branches in the medical control aspects of their programs. This is extremely important as much lost time and lowered productivity occurs as a result of uncontrolled environmental health hazards.

4. An evaluation of the reasons for employee visits makes it more and more apparent that the majority of employees' health problems are related in some manner to strained or poor human or interpersonal relations. More attention and staff research into methods of assisting employees in improving their interpersonal relations with other employees will be emphasized.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12171
SERIAL NO.

11. BUDGET DATA:

12. BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION []

REVIEW & APPROVAL [] TECHNICAL ASSISTANCE []

13. IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

Not applicable

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) WITHIN NIH)

PROJECT	EMPLOYEES REFERRED	REMARKS
1. NCI - Vaginal Cytology	1,405	3 cases of early cancer of cervix were diagnosed in symptom-free women
2. NIAID - Trichomonas Study	125	
3. NHI - Beta hemolytic strep control	408	
4. NIDR - Dysmenorrhea and oral pathology	50	

R.P.C. - 3 Analysis of NIH Program Activities
December 1955

Honors, Awards, and Publications Sheet

15. 12171
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CLAENDAR YEAR 1955

Not applicable

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Employee Health Service as a whole:

1. Certificate of Health Maintenance, Attesting to the High Standards of Medical Services Provided to Employees awarded to NIH during the American Medical Association's Congress on Industrial Health, held in Washington and Bethesda, January, 1955.

2. Invitation by American Industrial Hygiene Foundation to exhibit a series of panels, "Selected Occupational Health Services Available to NIH Employees" accepted and exhibit shown in Pittsburgh November, 1955.

John M. Lynch, M.D.

1. Declared Board eligible for new American Board of Occupational Medicine.

2. Appointed to Committee on Human Relations, Industrial Medical Association.

3. Elected to Fellowship, Industrial Medical Association

Frances S. Wolford, R.N.

1. Elected Secretary, Public Health Nursing Section, Graduate Nurses Association, Washington, D. C.

2. Appointed lecturer to Girl Scout groups on the Health Aspects of Puberty and Menstruation.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. The Clinical Center — INSTITUTE OR OTHER NIH UNIT
2. Office of Director — LABORATORY, BRANCH OR DEPARTMENT

3. Informational Services — SECTION
4. — LOCATION(IF OTHER THAN BETH.)
5. 12113 — SERIAL NO.

6. Informational Services — PROJECT OR ACTIVITY TITLE

7. Information Officer, Judson Hardy — PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. Not applicable — OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Informational Services continued to emphasize materials and activities designed to inform individual physicians and medical organizations concerning admission policies and diagnostic requirements of the various clinical studies. Two revised editions of the publication CURRENT CLINICAL STUDIES AND PATIENT REFERRAL PROCEDURES were issued and given wide distribution to individual physcians and medical organizations.

During the latter part of the year, a well-qualified editioral assistant was added to the staff and given primary responsibility for editing the transcripts of bi-monthly clinical staff meetings.

The volume of inquiries by telephone and letter processed by the office remained at a very high level during the year. A considerable number of inquiries were received from newspapers, magazines, radio and television sources.

Near the end of the year, Informational Services began working with the nursing Department and the Personnel Branch on the development of a greatly intensified nurse recruiting program.

At the request of The Chief of The Scientific Reports Branch, Information Services assumed an active role in the planning and conduct of tours, arrangements for the reception of distinguished visitors, and other activities of the NIH-Wide Special Events Program.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12113
SERIAL NO.

11. _____
BUDGET DATA:

12. _____
BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION []

REVIEW & APPROVAL [] TECHNICAL ASSISTANCE []

13. _____
IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Not Applicable.

14. _____
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not Applicable

R.P.C. - 3 Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. 12113
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

Project Description Sheet

1. The Clinical Center
INSTITUTE OR OTHER NIH UNIT

2. Professional Services Departments
LABORATORY, BRANCH OR DEPARTMENT

3. Medical Record Department
SECTION

4.
LOCATION (IF OTHER THAN BETH.)

5. 12133
SERIAL NO.

6. Administration of Medical Record Department
PROJECT OR ACTIVITY TITLE

7. Chief, Medical Record Department - Mrs. Gloria S. Burich
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. Chief, Research and Statistics Section - Miss M. Elaine Lynch;
Supervisor, Medical Transcribing Section - Mrs. Elizabeth Cavanaugh;
Supervisor, Records Control Section - Mr. H.G.W. Sundelof
Assistant Chief, Medical Record Department - Miss Jeanne G. O'Malley;
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Objectives:

1. To provide the service, maintenance and control necessary to assure accurate and complete records on all registered patients of the Clinical Center.

2. To supply clinical and research personnel with a wide variety of accurate statistical data that will enable each interested authorized investigator to have the advantage of information obtained from the analysis of medical records of all patients that have participated in studies of the Clinical Center; and to encourage the use of such data by making it readily accessible.

3. To provide for the long range collection of data for specific studies that may be of interest to one or more investigators in addition to the data routinely required.

Methods employed:

1. Institution of a recruitment and training program to insure the quality of employees required to provide for the accuracy demanded for the care of medical records.

R.P.C. - 1 (Cont'd) Analysis of NIH Program Activities
December 1955

Project Description Sheet

2. Assistance of Management Analysis Branch in conducting a survey of the entire Department to uncover those areas in which improvements could be made.

3. Implemention of procedural and organizational changes where the need is indicated.

4. Participation on the Medical Record Committee composed of representatives from each Institute. This committee, acting in an advisory capacity, meets monthly to appraise records and record systems and makes written recommendations to the Medical Board.

History:

The inpatient service reports revealed 1889 admissions and 1875 discharges during 1955. For each admission a history and physical examination averaging ten pages in length was transcribed and for each discharge a narrative summary averaging six pages in length was transcribed. Thirty-two employees were on duty in the Medical Record Department as of the last day of the year.

Comparative Summary-Inpatients:	1954	1955
Number of beds activated	332	396
Number of admissions	1452	1889
Number of discharges (includes deaths)	1326	1875

Major problems encountered:

1. Confusion of responsibility resulting from separation of the Medical Record Department from Admissions and Followup Department.

2. Lack of interdepartmental procedures and organization.

3. Difficulties in recruiting personnel (a) familiar with medical terminology qualified to transcribe with accuracy and speed, (b) with interest and ability in filing essential for the volume and specialized type of work which must be done by this Department.

Important progress or improvements achieved:

1. Recruitment and development of new section chiefs.

2. Initiation of a comprehensive survey to determine the interest of the clinical and research staff with the purpose of revising the reports offered by the Department and for increasing the scope of statistical data collected for special studies.

Project Description Sheet

3. Daily filing of patient records in the program areas to reduce the delays in filing of miscellaneous reports and the frequency of recalling charts.

4. Improvement of the locator files for all medical records and the preparation of a more complete monthly report on incomplete records.

Proposed Course of Activities during the next calendar year:

1. Enlarge scope of statistical data offered for special studies.

2. Continue re-evaluation of routine reporting system and adjust to what is indicated as being greatest value to the staff.

3. Convert all medical statistical data to IBM.

4. Transfer remaining activities appropriate to the Admissions and Followup Department. e.g. - pre-admission correspondence.

5. Initiate an intensive training program in all aspects of filing and handling of medical records and in medical terminology for transcribing so that when there is a turnover in the Medical Record Department, qualified replacements will be available to avoid the backlog of critical material which develops during a period of recruitment to fill vacancies. In addition, Clinical Departments will be encouraged to recruit personnel from the transcribing pool on the rationale that when such trained persons are placed throughout the Clinical Departments the control procedures of the Medical Record Department will receive better cooperation and to utilize existing training and experience of Medical Record Department.

6. Installation of Soundex filing cabinet that will accommodate standard-size addressograph cards thus eliminating typing and duplication of files.

7. Employment of additional features of Addressograph equipment in order to utilize it to its full capacity.

8. Relieve crowded sections of the Department by removal of partitions and rearrangement of personnel and improvement of equipment.

9. Development of Standard Operating Procedures.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12133
SERIAL NO.

11. BUDGET DATA:

12. BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION []

REVIEW & APPROVAL [] TECHNICAL ASSISTANCE []

13. IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Not Applicable.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not Applicable

R.P.C. - 3 Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. 12133
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Clinical Center
INSTITUTE OR OTHER NIH UNIT

2. Professional Services Department
LABORATORY, BRANCH OR DEPARTMENT

3. Nursing Department
SECTION

4. _____
LOCATION (IF OTHER THAN BETH.)

5. 12141
SERIAL NO.

6. Nursing Activities
PROJECT OR ACTIVITY TITLE

7. Ruth L. Johnson, Chief Nursing Department
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. Louise C. Anderson, Ass't Chief, Nursing Department
Josephine I. O'Connor, Ass't. to Chief, Nursing Department
Gwen T. Will, Chief, Psychiatric Nursing Service
Elizabeth Walker, Chief, Cancer Nursing Service
Jane Wilcox, Chief, Heart Nursing Service
Louise C. Anderson, Acting Chief, Neurology and Blindness Nursing Service
Margaret A. Benson, Chief, Infectious & Tropical Diseases Nursing Service
Marie M. Ceglarek, Acting Chief, Arthritis and Metabolic Diseases Nsg. Service
Janet Fitzwater, Chief, Surgical Nursing Service
Odile M. Morneault, Education Officer
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Objectives:

A. To cooperate in planning for and in rendering service for the admission, clinical care, research activities and followup of patients admitted to the Clinical Center.

B. To provide a high standard of nursing care for patients and assistance to the clinical research staffs in the categorical Institutes.

C. To foster the research point of view among members of the nursing staff through better understanding of the goals of the clinical research projects.

D. To carry out a staff education program which will enable nursing personnel to function most effectively in the patient care program and help them to derive satisfaction from their work.

E. To cooperate with all departments and services in working toward the goals of the Clinical Center.

Project Description Sheet

F. To improve methods of diagnosing and dealing with nursing problems through the group process.

G. To improve communication within the Nursing Department and between the medical and nursing staffs.

H. To improve the attendant training program.

Major Problems Encountered:

1. The major problem has been a lack of sufficient applicants for staff nurse GS-5 and GS-7 positions, the poor quality of the applicants for the GS-9 and higher grade positions, and the poor quality of the applicants for hospital attendant GS-2 positions. We have carried vacancies at all levels the whole year and have found that we have barely been able to keep up with the turnover of personnel.

2. The effective utilization of the nursing personnel on duty has not always been possible for a number of reasons:

 a. There has been a lack of sufficient advance planning on the part of some of the investigators with the nursing staff for activities which require considerable nursing participation.

 b. Unexpected admissions which require many adjustments in order to find a suitable place for the patient. This is often further complicated by conflicts among the investigators in regard to the number of beds each has on a unit or service.

 c. There have been marked variations in the surgical schedules so that planning for nursing coverage has been difficult. On the heart service it has varied from many major procedures in one week to none the next week. This has resulted in many time changes for personnel in order to cover when the schedule was heavy.

 Cancellation of surgical procedures already scheduled and addition of procedures to the operative schedule without sufficient advance notice to both the patients and the nursing staff has created problems in the nursing units as well as the operating rooms. (See, also, report from Surgical Nursing Service.)

 d. A great deal of time is spent by nursing personnel in staying with patients in the diagnostic X-ray Department, Radiation Therapy, Radioactive uptake laboratory and Photographic Department. With the increasing number of patients seen in these areas, each department should now plan to provide for these

services within their own departments. The number of nursing personnel involved and the amount of time spent has often deprived the nursing units and this has been reflected in inability to meet the needs of patients and the medical staff. The nursing services cannot carry this load much longer and some other arrangements will have to be made.

3. The nonsegregation of children from adult patients; lack of facilities for the care of patients who develop psychotic behavior; lack of bath and showering facilities for wheel chair patients on all services and lack of bathtubs on the Infectious Diseases Service (11W) are still problems which have not been solved.

Progress or Improvement:

1. The new qualification standards and job specifications for the Nursing Assistant Series have been published by the Civil Service Commission. This should help us in our recruitment and selection of nonprofessional personnel.

2. A recruitment program for professional nurses is underway and this should help us in filling our nurse position vacancies.

3. The training program for Nursing Aides has been revised and extended. A 40-hour training period for Ward Clerks has been completed and put into effect.

4. Service Supervisors on the afternoon and night tours of duty have been appointed and are functioning on the Cancer, Heart, Neurological and Psychiatric Nursing Services. This has resulted in greater continuity of patient care and personnel supervision.

5. The schedule of clinics in the Admissions and Followup Department has been readjusted and this should make it possible for the nursing staff to g ve better care to patients and assistance to the clinicians.

6. Professional Nurses are now being employed on a part time basis (through WAE appointments) to supplement the full time staff. These nurses carry a great deal of the continuous nursing care to patients who require it and also are available to replace staff members who are on sick and annual leave.

7. Staff development programs on all services have been better planned and have created much interest on the part of the nursing personnel. We are in a much better position to provide a high caliber of experience to graduate nurses who wish to specialize in any of the services available here.

8. There seems to be a better understanding of the role and functions of nursing on the part of the medical staff and others and this has resulted in better working relationships and a more cooperative spirit.

Significance of Activities to the Clinical Research Program:

The research programs on all services are not viewed as something apart from, but as an integral part of the day-to-day activities. The nurses's participation in research is two-fold: (1) She provides nursing care to patients who are under study. This involves meeting all their needs whether physical or emotional so that they are able and willing to take part in the research programs and remain as long as they are needed. (2) She participates as a member of the research team by collection of data, recording of observations, collection of specimens, assisting in designing equipment, assisting in planning procedures and writing of manuals. Her part in the clinical research program is often cruical to the investigative staff and every effort is made to make this clear and understandable to her through our staff education program.

Nursing Research:

A number of studies have been made which have aided in better utilization of staff and which have revealed staffing needs on the various services. These have been done as part of the overall administrative process and probably cannot be considered true research. It is hoped that we can go forward with some planned projects during this calendar year. In preparation for this, a weekly research seminar is being held each Wednesday from 5 p.m. to 6:30 p.m. Attendance is on a voluntary basis and is open to any nurse who is interested.

Objectives for 1956:

The objectives for 1956 are the same as for 1955 with the following additions:

1. To develop field experience programs for graduate nurses.

2. To begin research in nursing - particularly in the area of patient nursing needs.

3. To write for publication in the professional journals.

R.P.C. - 1 (Concl'd) Analysis of NIH Program Activities
December 1955

Project Description Sheet

Addendum:

A. Number of Personnel on duty

	January 1, 1955	December 31, 1955
Professional Nurses	221	257
Practical Nurses and Hospital Attendants	109	150
Clerical Staff	26	32
Total	356	439

B. Overall average number of nursing hours available per patient per day for 1955 was 5.82

Average number of nursing hours available per patient per day for each Nursing Service during 1955 was:

Arthritis and Metabolic Diseases	5.50
Cancer	5.79
Heart	5.13
Infectious and Tropical Diseases	7.28
Neurology and Blindness	4.9
Psychiatry	8.01

C. Number of hours of continuous nursing care (special nursing to individual patients) during 1955 was:

Arthritis and Metabolic Diseases	1740
Cancer	5419-1/2
Heart	4348
Infectious and Tropical Diseases	9294
Neurology and Blindness	12387
Psychiatry	2323-1/2
Total	35512 hours

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12141
SERIAL NO.

11. BUDGET DATA:

12. BUDGET ACTIVITY:

RESEARCH (SERVICE)	[X]	ADMINISTRATION	[]
REVIEW & APPROVAL	[]	TECHNICAL ASSISTANCE	[]

13. IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

Not applicable

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH)

Not applicable

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. 12141
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Not applicable

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Not applicable

Analysis of NIH Program Activities

Project Description Sheet

1. Clinical Center — INSTITUTE OR OTHER NIH UNIT
2. Office of Director — LABORATORY, BRANCH OR DEPARTMENT
3. Office of Director — SECTION
4. — LOCATION (OTHER THAN BETHESDA)
5. 12115-12111 — SERIAL NOS.
6. Office of Director — PROJECT OR ACTIVITY TITLE
7. D. W. Patrick, M. D., Director
Stuart M. Sessoms, M. D., Assistant Director
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY
8. Margaret C. Hannigan, Patient Librarian
Rev. William R. Andrew, Chaplain
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS
9. PROJECT DESCRIPTION

During 1955, a total of 1,875 inpatients were admitted and discharged as against 1,326 during the preceding year. The average daily census was 264 as compared to an average daily census of 176 in 1954, and the average length of stay per inpatient was 48.33 days, as contrasted with the 40-day average last year. The average daily percentage occupancy of total activated beds was 71.91%, the corresponding figure for the previous year being 72.15%. Details of bed activation and patient census by Institute are contained in the following table.

TABLE I

Institute	Total Bed Complement		Patient Census	
	Jan. 1	Dec. 31	Jan. 1	Dec. 31
NCI	76	91	48	64
NIAID	36	39	29	19
NIAMD	48	52	32	20
NHI	62	72	36	43
NINDB	52	67	40	49
NIMH	58	73	29	34
NIDR	-	2	-	2
TOTAL	332	396	214	231

As was anticipated; a large proportion of the patients were referred by physicians in nearby states, classified according to Institutes as given in detail in Table II.

TABLE II

DISTRIBUTION OF DISCHARGED INPATIENTS
BY INSTITUTE AND GEOGRAPHIC REGION
January- December 1955

AREA	INSTITUTE							
	NCI	NHI	NIAMD	NIMH	NMI	NIMDB	NIDR	TOTAL PATIENTS
REGIONS								
New England	2	7	3	2	1	7		22
Middle Atlantic	9	27	43	15	2	45	1	142
East North Central	9	36	16	10	3	15		89
West North Central	2	10	6	6		15		39
*South Atlantic	394	351	181	46	240	240	3	1455
East South Central	12	28	10	2	11	12		75
West South Central	5	5	2		5	6		23
Mountain	1	3	1			2		7
Pacific		3	2	3		5		13
U.S. TERRITORIES						2		2
CANADA	1			1				2
OTHER NON-U.S.		2		2	1	1		6
Total Patients	435	472	264	87	263	350	4	1875
*								
District of Columbia	139	116	55	28	73	58	2	471
Maryland	132	133	65	15	129	73		547
Virginia	73	69	39	1	29	76	1	288

The scope and importance of followup activities continued to increase. This phase of the program is described in more detail in the separate report from that department.

The occupancy rate for the year for beds actually opened has been lower than anticipated. This has been due to the unforeseen requirements developed by research studies, which in turn required changes in plans to meet the increased demand for nursing services.

Inability to recruit enough nurses has also forced us to delay the activation of some new beds. Although the nationwide shortage of nurses has existed for some time, it was not until this year that it became so serious as materially to effect our plans for activation of beds and a continued orderly increase in the number of research patients admitted. This effect was noted particularly in the last quarter of the year. As soon as this situation became apparent, a strongly reinforced program of nurse recruitment was initiated. The increased recruitment of nursing personnel plus two other developments have begun to alleviate the shortage.

Increased use of part-time nurses has temporarily added to available nursing hours. Simultaneously, changes in research program emphasis have reduced the number of patients requiring continuous intensive nursing care. These measures have permitted a diffusion of available nursing hours among a greater number of patients.

A new nationwide Civil Service examination for nurses will be announced in the near future. Past experience indicates this will bring an immediate and sizable increase in the number of qualified nurses available for appointment. A full time nurse recruiter has been added to the staff and a descriptive recruitment brochure will receive wide circulation throughout the nursing profession. The medical and research staff have become increasingly aware of the nursing shortage and are utilizing every opportunity in the course of their professional contacts to attract nursing personnel into their respective programs.

MEDICAL BOARD

The Medical Board for 1955 had a new chairman and two new members. There were a number of changes in appointments to the various committees of the Medical Board; some were due to the enlargement of several of the committee memberships.

A Nursing Committee was established. The function of this committee is to make recommendations to the Medical Board regarding policies and procedures which jointly concern the medical and nursing staffs in the care of patients.

The Clinical Research Committee has been active this year principally because of the number of projects involving normal volunteers, as it is mandatory that all of these projects be referred to the Clinical Research Committee.

The Organization and By-laws Committee held a series of meetings to revise and bring up to date the Organization and By-Laws document with respect to suggestions that were made following the revision dated March 16, 1954, and also with regard to Medical Board actions since that date. This revision is near completion, at which time it will be circulated to the Medical Board members for review and adoption.

The Radioactive Isotopes Committee received a Broad License for the use of radioactive isotopes in human applications, dated November 10, 1955. Under the general provisions of this authority, the Radioactive Isotopes Committee assumes entire responsibility for the safety of patients and normal human subjects in which radioactive materials are used for diagnostic, therapeutic and research purposes. The license includes all radioactive isotopes from atomic number 3 to 83, with the exception of Sr.

During the first year and a half of the Medical Board's operation, the principal function was along organizational lines but that is now changing, and in the future the Board will be able to devote more of its time to operational problems.

PANEL OF CONSULTANTS

During the year 1955 there were 553 visits by medical care consultants to the Clinical Center. Sixteen medical care consultants have been added to the roster, the total number now being 158.

The system for calling medical care consultants has been satisfactory. Institute staff members responsible for covering a particular specialty are designated as panel chiefs. If a staff member wishes the services of a medical care consultant he makes known his desire to the responsible panel chief who then makes arrangements for securing the services of the consultant desired. In this manner a consultant can be used by more than one Institute during one visit to the Clinical Center. It is felt that the consultant program as a whole has worked out very well.

PATIENT LIBRARY

Patient library services continued its role in the maintenance of patient morale, and in some instances, contributed to the therapy of individual patients. An important adjunct to the full time professional librarian was the assistance provided by the Red Cross Gray Ladies who worked a total of 2,376 hours.

The Librarian conducted two twelve-hour in-service training courses for the Gray Ladies and lectured on patient library service each week to new members of the Nursing Department staff. The number of catalogued books in the library at the end of the year was 1,909, nearly double the total at the end of the preceding year. Over one half of acquisitions were gifts.

During the year frequent use was made of mechanical aids and special materials such as talking book machines and records, ceiling picutres, books on film, linguaphone records and Braille books. The average weekly circulation of books from the library was 144. Extensive use was made also both in the Library and on patient units of daily newspapers and magazines. Continuous coordination of librarian service with the school program, a readers' advisory service, story hours and special reading program, and individual work with patients at the request of physicians were other features of the year's activities.

CHAPLAIN'S SERVICE

It was recognized during the planning of the Clinical Center that patients for medical and psychiatric research are faced with the emotional and spiritual crises of chronic and terminal illnesses. To meet these spiritual needs, plans were made for an appropriately furnished chapel and chaplaincy services. Beginning in 1950, consultations were held with the Washington Federation of Churches, the Archdiocese of Washington, and the Washinton Board of Rabbis.

In 1953 a plan was adopted to provide a full time Protestant chaplaincy and part time Roman Catholic and Jewish chaplains, based on the ratio of patients of each faith group (75%, 18% and 7%). A job description and civil service classification for a Protestant "Supervisory Chaplain" was accepted by the Administration and Personnel Department, with request that the Washington Federation of Churches nominate qualified candidates for the position. In the interim a plan was effected to provide religious services and pastoral visitation under "Non-personal Services Contracts." In October 1955 a full time Supervisory Chaplain was appointed by the Clinical Center and officially installed by the Washington Federation of Churches, December 1955.

The duties of the Supervisory Chaplain are: administrative coordination of all religious activities; Protestant worship services; bed-side ministry to new, surgical, seriously ill and specially referred patients and their families; meetings and consultations with the administration staff and the staffs of the seven Institutes; counseling employees; interpreting the Clinical Center to church and community groups; research in ministry to the sick. His immediate goal is extensive orientation to the patients and the research programs with respect to the patients' needs and comforts, in order that he may define a more realistic chaplaincy program, with necessary personnel, to meet the special requirements of the Clinical Center.

The Catholic Chaplain serves an average of twenty hours per week, including regular and special Masses, and rites, sacraments and pastoral care for individual patients. The Jewish Chaplain makes pastoral calls and arranges attendance at the nearby synagogue.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12115-12111
SERIAL NOS.

11. BUDGET DATA:

12. BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION []

REVIEW & APPROVAL [] TECHNICAL ASSISTANCE []

13. IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Not Applicable.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not Applicable.

R.P.C. - 3 Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. 12111-12115
SERIAL NOS.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Clinical Center — INSTITUTE OR OTHER NIH UNIT
2. Nutrition Department — LABORATORY, BRANCH OR DEPARTMENT
3. Patient Dietetic Services; Food Production and Cafeteria Services — SECTION
4. LOCATION (IF OTHER THAN BETH.)
5. 12151 thru 12155 — SERIAL NO.

6. Clinical Center, Nutrition Department, Annual Report — PROJECT OR ACTIVITY TITLE

7. Edith A. Jones, Dietitian Director, Chief, Nutrition Department — PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. Ann Reimer, Sr. Dietitian (R), Chief, Patient Dietetic Services
Margaret Vance, Sr. Dietitian (R), Chief, Food Production and Cafeteria Services — OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Objectives:

A. To provide within a given budgetary framework all nutrition services to all patients in the Clinical Center as ordered by the physician in charge of each project, to meet the research program needs of the seven National Institutes of Health.

B. To provide also a food service on a "self-supporting basis" to the personnel engaged in the research activities of the National Institutes of Health as well as provide special arrangements for groups desiring to eat together in group meetings.

C. To provide diet therapy instruction to in-patients, to patients being discharged and to out-patient research patients upon request of physician in charge.

D. To conduct research in clinical nutrition and nutrition service administration.

E. Attachment I contains a more detailed information.

Methods Employed:

A. Operated a Main Kitchen approximately 14 hours daily to furnish food needs to all patient areas and to Cafeteria for personnel.

B. Employed decentralized food service throughout the Clinical Center with the use of 12 Floor Kitchens in operation at the end of the year 1955. This included responsibility for total food service including delivery of trays to patients and collecting trays as well as serving morning and afternoon nourishment.

C. Operated a formula room service to all 12 Nutrition Units.

D. Made available technical nutrition service and information to the participating physician when requested and ordered.

E. Two Metabolic Research Kitchen Units were operated throughout the year, providing constant diets to those patients on complete balance studies from the National Heart Institute, and National Institute of Arthritis and Metabolic Diseases, and a minimum number of patients from the National Cancer Institute.

F. A "pay cafeteria" was operated with food service available 11 hours daily, between the hours 7:30 a.m. and 6:30 p.m. on week-days, and open on holidays and weekends for three meal hours only.

G. For more details, see attachment II, and also attachment entitled "Guidelines of Food Service of the Nutrition Department."

Numbers and Kinds of Major Services Rendered:

A. Main Kitchen

From January 1 to December 31, 1955, the Main Kitchen provided cooked food for the distributed food to 12 Floor Kitchen Units and provided raw food to 2 Metabolic Research Kitchen Units for 282,036 patient meals. Also, food was provided for sale in the Clinical Center Cafeteria for 604,724 customers within the year. (See table VI for analysis of cafeteria sales and table I for analysis of patient meals.)

B. Of total meals served to patients January 1, 1955 to December 31, 1955: 282,036.

Serviced by Institutes, approximately:

21% to NCI
21% to NHI
8% to NMI
13% to NIAMD
16% to NIMH
21% to NINDB

(See attachment, Table I, for tabulation.)

C. Modified Diets, January 1, 1955 to December 31, 1955.

Of the meals served in the Floor Kitchens (269,182 meals), approximately 46% were modified diets (special). This represents all diets served other than normal, soft and liquid diets. (See Tables II and VI for tabulations.)

D. Weighed Modified Diets Served:

Of the total number of modified (special) diets served from the Floor Kitchen Units, the needs for weighed diets by Institutes were as follows (See Table V for tabulations.):

NCI	35%
NHI	42%
NMI	12%
NIAMD	28%
NIMH	3%
NINDB	5%

E. Of the total meals served to the patients within the year, only 25 - 30% were provided as normal food service - the remaining were provided as a supporting service to specific research projects and, in many cases, were therapeutic in value also.

F. Needs supplied specifically to the Mental Health Program and Patients:

In order to meet program needs of the Mental Health Floors, an essential part is the plan for meal activities other than the typical family style patient meal service. The Nutrition Department Provides television lunches, picnic baskets, food packed for "cook-outs," refreshments for birthday parties, popcorn balls, and candy making parties, as well as packed food supplies for camping trips. Within the last year these activities have averaged three to four times each week.

G. Test meals and services to Follow-up, Occupational Therapy, Pharmacy Department, and Blood Bank.

Although minimum in number, the Nutrition Department makes a limited supply of food items for the following reasons:

a. To the Blood Bank to be served to the donors.
b. To the Occupational Therapy Department to be used in their therapy program.
c. To the Pharmacy for such items as they may need in the preparation of some medications.
d. To the X-ray Department for test purposes.
e. To the Follow-up Department for those patients who are unable to go to the Cafeteria, and who must remain over the noon meal period.

H. The Formula rooms daily services have averaged:

12 - 14 regular tube feedings
2 - 3 metabolic tube feedings for specific studies
2 - 5 Baby formulas with water, orange juice
2 - 7 Quarts, eggnog
3 - 4 Quarts, reaction juice
8 - 12 Quarts, fortified milk formula

I. Meals served by Research Kitchen Units:

12,844 meals were served as constant diets as a part of balance studies engaged in by National Institute of Arthritis and Metabolic Diseases and National Heart Institute and National Cancer Institute. This represents approximately 5 per cent of the total meals served in the Clinical Center within the year. (See Table III for tabulation.)

J. Cost of Food Service: See Table VI

a. Raw food

Within the past 12 months, the raw food costs of the Nutrition Department have fluctuated from $1.35 per ration to $1.60 per ration served. It is obvious from Table VII that the fluctuation is small and could not be considered significant in light of changes of local food prices, but the fluctuations are indicative of the quantity and kind of modified diets that are ordered for research patients.

As indicated elsewhere in this report, a large percentage of the rations served by the Nutrition Department in the Clinical Center are modified diets which are a very pertinent part of the research program. Modified diets in this figure means those rations that are planned by a highly trained professional staff to meet specific research needs of the individual projects of the various Institutes. Typical examples of such diet orders would be to give increased quantity of protein, or restrictions to various sodium levels, (lower levels being more costly than the more liberal levels), controlled constant diets to be served from the floor kitchen units, and in many cases in our cancer studies, special prepared liquid feedings are necessary in order to fulfill the needs of the patient as well as the needs of the research study.

In order to meet the requirements of constant diets such food items as beef tenderloin, the eye of lamb chop, and breast of white chicken are the restricted meat items that can be used on these diets, which in themselves average more than one-third of the cost of a daily ration. Also the special liquid feedings, because of the special protein preparations that are necessitated, range in cost from $1.75 to $2.25 per day per patient.

Diets of constant items are very often ordered from the Floor Kitchen Units and this involved preparing simple food items but items that often are not "the cheap items of the food market and may not always be in season." At the same time for many of these diets and others served from the Floor Kitchen Units, it is necessary for the investigator to have a duplicate tray of what the patient has eaten to be analyzed in his laboratory for comparison sake. This consequently represents in many cases that the raw food ration for one patient is doubled.

Also, to meet with the program needs of the Mental Health investigators, we routinely allow two portions (in quantity) of food items on the menu (per patient). This is as much a part of their research program as a low sodium diet might be to another Institute. This is significant in that this represents approximately 20% of the diets shown as Normal Food Service Diets.

In the Metabolic Kitchen Units, the same fluctuation in food costs occurs with the various types of diets that are requested. Experience in 1955 indicates a variation in the Metabolic Kitchen Unit cost from $1.38 per ration to as high as $2.28 per ration. A review of the detailed records indicates here again that the difference in kind of specified constant diets ordered for research purposes has necessitated unusual items to be used to fulfill the nutrient needs that the investigator has ordered.

b. Service and Handling

As shown in Table VII, this represents the greatest portion of our costs - averaging 79%. Broken down this means 76 - 77% goes into personnel program, and 2-3 % into dishwashing powder, paper supplies, etc.

As can also be seen in this table, the additional floor kitchen opened and a low occupancy rate controls the ration costs. During 1955 we maintained an occupancy rate of 71%, and an average ration cost of $6.88. It is estimated that had we maintained an occupancy rating of 80%, our average ration cost might have been $6.26.

K. Patient Instruction:

There are an increasing number of patients being discharged with specific dietary instruction from the various Clinical Center services. This involves approximately 10 per cent of the total patients discharged.

Patients seen in Follow-up Department for dietary instruction amount to approximately 10 - 15 visits per month.

L. Cafeteria Service:

604,724 sales were served throughout the year. This has been an increase from an average of 12,576 per month.

The above represents $245,191 collected in receipts for the entire year.

The year has seen a gradual increase in the number of customers served daily as well as on weekends. We now serve an average of 2,100 daily (Monday through Friday) and 500 - 600 on Saturdays and Sundays.

This year has also seen an increase in the average sale from an average sale of 40¢ to 41¢ per sale.

At the end of the fiscal year 1955, the Cafeteria showed a profit of $5,874 representing less than 2-1/2 of the total sales of the year.

Summary: The raw food cost in the Cafeteria has ranged from 35 - 48% of the total $1.00 received: and the personnel costs have ranged from 38 - 51% of the total $1.00 received. We have arranged - raw food, 40%; personnel, 44%. (See Table VII)

Special Groups - Eating arrangements are made and reserved for groups wishing to eat together in the Cafeteria or Hospitality and Snack Shop for business reasons. This averages arrangements for one special group each week day.

M. Food Purchase:

a. Specifications and continuing contracts

Specifications are continually reviewed and usage figures are prepared and a continuing contract is requested for eight groups of food items each month. This includes monthly contracts for: Meats (beef, pork, lamb, veal), coffee, poultry, fresh fruits and vegetables, fish, frozen foods, and cake flour. Specifications for contracts for longer periods of time for milk, ice cream, bread and crackers, are requested through the Nutrition Department.

b. Revolving Fund

Approximately 160 food items (canned goods and staples) are still under the Revolving Fund of Purchase and Supply Branch and assistance given in developing specifications and making decisions as to items to be purchased and stocked in the Warehouse.

c. Other food items

Other food items not included under either of the above two are requested routinely with accompanying specifications and source of supply.

d. Bills Cleared for Payment

Nutrition Department assumes full responsibility for checking all delivery tickets accompanying food items ordered and received against contract prices and obtains and prepares all necessary information for final payment on food stuffs furnished the Nutrition Department.

N. Food Cost Accounting:

Basic data collected representing:

a. Pricing and extending on daily requisitions from Food Production.
b. Daily summary sheets with cost as to food groups.
c. Control register maintained of food and supply purchases.
d. Periodic physical inventory made plus check with control register.

Important Progress or Improvement Achieved

A. A complete review of the specifications and procedures used by the Supply and Management has improved the quality and service of the Nutrition Department.

B. Within this year three additional floor kitchen units have been opened to service the needs of the clinical research program.

C. Additional staff have been added to the two Metabolic Units in order to be able to service approximately 10 balance studies from each of the Metabolic Kitchen Units.

D. Complete job review and all jobs were redescribed for entire Department in order to facilitate the transfer to Wage Board Scale.

E. Civil Defense Plan for feeding in case of disaster was revised for use at Clinical Center and participated in drill.

F. Provided Nutrition Services as requested and indicated in Parts I, II, and III.

G. With a change in programs in the National Heart Institute, a need developed for the use of oral liquid formulas, to be prepared in one mixing, an amount for two months period, which necessitated deep freeze storage also. This process has been worked out and with

the assistance of the Pharmacy Department, a satisfactory service rendered. At the recommendation of the Medical Board's Nutrition Committee, plans are underway to extend the facilities so that total process can be completed by the Nutrition Department within one area.

H. It has been necessary to reorganize and expand the accounting unit and as a result, daily cost operations are available as well as perpetual inventory cards are maintained so that a large number of our food staples are automatically reordered when they reach a given level.

I. An employee manual for orientation and reference has been developed for use in the Nutrition Department.

J. Extensive figures have been collected throughout the year in order to develop accurate meat factors to be applied in the accounting operation in order to account for financial losses in shrinkage, bones, etc.

K. Storeroom facilities in the Main Kitchen are limited. However, within the year, there has been considerable reorganization and each storeroom area arranged in alphabetical order and, at the same time, preprinted requisitions were put into use. Also, we began placing daily orders for staple supplies for those supplies stocked in Building 13 under the revolving fund. All of these resulted in a more efficient operation.

L. Staff dietitians attended the following and reported on information presented at each to the total staff:

1. Course in Food Technology given by Massachusetts Institute of Technology, Boston - 6 weeks.
2. American Hospital Association Institute of Dietary Department Administration - 1 week.
3. Nutrition Symposium - 1 day.

M. The following new equipment has been installed and has been a real help in improving our service.

1. Plastic dishes in use on 5 floor (for Mental Health and NINDB patients).
2. Six ice cream storage cabinets installed with one in every other floor kitchen.
3. A rinse injector installed on the main dishwashing machine for cafeteria use. This has assisted with "quick drying" of silver and plastic trays as well as glassware and china.

N. A review of the activities of the total staff indicate that 23 nonprofessional and 6 professional staff have been enrolled in outside classes during the year.

SIGNIFICANCE OF ACTIVITIES TO THE CLINICAL RESEARCH PROGRAMS

A. As indicated in this report, the Nutrition Department has provided considerable supporting services to the clinical research programs of each of the Institutes of the National Institutes of Health as well as provided technical nutritional information as needed in these research programs.

B Essential to accomplishing the clinical research programs is satisfied personnel. It is felt that the food service to personnel is an important adjunct to this.

PROPOSED COURSE OF ACTIVITIES DURING NEXT CALENDER YEAR

A. Completion of construction of new formula room to house operations now temporarily located in the 8th Floor Metabolic Kitchen Unit.

B. After formula room, revise the 8th Floor Metabolic Kitchen Unit to provide for oral liquid metabolic studies.

C. Opening of one additional floor kitchen unit.

D. Opening the Hospitality and Snack Shop for extension of services of the National Institutes of Health personnel in evening hours.

E. Extension of additional items to be covered under the Revolving Fund.

F. Review and revise all job descriptions and routines.

G. Conduct an intensive training program for all nonprofessional and professional staff.

ACTIVITIES INVOLVING COOPERATIVE RELATIONSHIPS

A. Chief of the Nutrition Department serves as a member of the Nutrition Advisory Committee appointed by the Medical Board.

B. Chief of Nutrition Department serves on Commissioned Officer Board for dietitians throughout the Public Health Service.

C. Nutrition Department was asked to demonstrate its activities and practices to the following groups:

1. University of Maryland students (dietetic majors)
2. Dietetic interns - Walter Reed General Hospital
3. Dietetic interns - Freedman's Hospital
4. Graduate students - Georgetown School of Nursing
5. School of Hospital Administration - Naval Medical Center
6. Graduate students on Community Nutrition - University of North Carolina.

D. Three - four members of the Nutrition staff serve each month on a Civil Service panel for rating applications of nonprofessional employees to the Board of Civil Service Examiners.

E. Chief of Nutrition Department acts on Board for making appointments for dietetic internships to the Staten Island Hospital.

F. There are continuous cooperative studies undertaken by the Nutrition Department and the Sanitary Engineering Branch of the National Institutes of Health in areas that affect the operation of the Nutrition Department.

G. Assistance has been given in reviewing the layout and equipment ordered for the Clinical Center with two different State Health Department Dietitian Consultants this year in order to give them assistance and the benefit of our experience.

H. A guest employee, Mrs. Inger Sondergaard, from Denmark, was in the Nutrition Department until February to observe the work of a hospital dietitian in the United States.

I. Three members of the Nutrition Staff were able to attend the Annual Convention of the American Dietetic Association in October.

J. Consultation has been given to Indian Health Service and United Mine Workers on developing their Nutrition Program.

K. Assisted with Diet Manual - Hospitalities Facilities Division

L. The Nutrition Department has assisted with pictures and story material about the Department to be published in assisting with the Nurse Recruitment Program.

M. Assisted Sibley Hospital with its plans for nutrition facilities in its new building.

N. The Nutrition Department provided summer practicing experience for four college students majoring in dietetics.

NOTE: Because of their length, charts have been omitted. They may be consulted in the Office of the Director, Clinical Center.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12151 thru 12155
SERIAL NO.

11. BUDGET DATA:

12. BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION []

REVIEW & APPROVAL [] TECHNICAL ASSISTANCE []

13. IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S).

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH.

None

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. 12151 thru 12155
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

The Dietitian and Her Job - Trustee, October, 1955 - Edith A. Jones

Is Research Necessary for Equipment and Layout Planning - Journal of the American Dietetic Association, September 1955 - Edith A. Jones

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

A. The Chief of the Nutrition Department was elected President, The D.C. Dietetic Association for the year 1955 - 1956. This is an unprecedented second term. At the same time, she was elected Delegate for this group to the National Convention.

B. In October the Chief of the Nutrition Department was invited to speak at the Annual Meeting of the American Dietetic Association in Atlantic City. Her subject was "Dietitian-Patient-Hospital Staff Relationship."

C. The Chief of the Nutrition Department served as chairman of the registration of the Military Surgeon Annual Meeting held here in Washington, D.C.

D. Six staff members participated in the Annual Tri-State Hospital Meeting held here in Washington, D.C.

E. Staff Dietitian in Food Production is currently serving as Chairman of the Professional Education Committee of the District of Columbia Dietetic Association.

F. Staff dietitian in Patient Dietetic Area, Miss Gamble, is currently serving as Chairmam of the Diet Therapy Committee of the D.C. Dietetic Association.

G. A staff dietitian, Miss Turrentine, was asked to serve on the evaluation committee for the Annual Convention of the American Dietetic Association.

H. Another staff member is currently serving on the Community Nutrition Committee for the American Home Economics Association.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Clinical Center — INSTITUTE OR OTHER NIH UNIT
2. The Pharmacy Department — LABORATORY, BRANCH OR DEPARTMENT
3. Pharmacy Service / Sterile Supply Services — SECTION
4. — LOCATION (IF OTHER THAN BETH.)
5. 12130 — SERIAL NO.

6. Pharmacy Service and Sterile Supply Service
PROJECT OR ACTIVITY TITLE

7. Milton W. Skolaut, Chief, Pharmacy Department
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. Joseph N. Salvino, Chief, Sterile Supply Service
John A. Scigliano, Ph.D., Assistant Chief, Pharmacy Department;
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Objectives:

To supply to the clinical research teams all necessary pharmaceuticals and sterile supplies. To provide consultation and advice on problems encountered and produce or process new developmental forms of dosages, oral or injectable, and trays or equipment essential to the successful completion of a study.

Methods Employed:

A coordination of pharmaceutical, sterile supply and allied needs under one responsibility. Establishing fast and efficient methods to obtain new and special supplies with the Supply Management Branch. Advisory functions of the Pharmacy Committee and the Nursing Procedure Committee have been very helpful.

Major Services Rendered:

Total Number of Department Item Issues	1,327,637
Total Number of Individual Requisitions Processed for the Department	22,116

Pharmacy Service Item Issues

Prescriptions	7,571
Other Items	142,940
Total	150,511

Number of Individual Requisitions Processed for Other Items		9,381
Sterile Supply Service Item Issues		
Sterile	825,004	
Clean	352,122	
Total	1,177,126	
Number of Individual Requisitions Processed		12,735
Number of Equipment Loans Processed		1,957

The Pharmacy Service issues can be placed in two categories, routine and special. Certain functions are rather hard to place clearly in just one category. Routine items in the Clinical Center are classed in this manner only because of repetition in dispensing. A great portion of these items would be classed as special in the ordinary hospital environment.

Special item issues include new preparations which are being made for the first time, new dosage forms of available drugs and unusual combinations. These preparations include injectables, tablets (coated and plain), capsules, oral suspensions, ophthalmic medications, powders and oral preparations. These preparations are very time consuming since they require planning, obtaining of the materials, trial preparations, actual preparation, packaging and testing. The need for special items has been increased four fold from 1954. This is the area in which the Department can offer the most valuable service. In this area the staff will have to be increased as the demand for services increases.

The Sterile Supply Service has increased the processing of "Special Handling" items. These materials have been processed apart from the routine items since they have been specially made, modified or treated. The surgery supplies are now prepared by personnel of our Department. Close coordination and cooperation with the Surgery Nursing personnel results in excellent training of our personnel in this particular function by a Surgery Nurse. This has resulted in excellent supplies being furnished to the specialized Surgery Service and has released professional nurses.

The Department has accomplished or cooperated in the following services:

a. Furnished two hours of orientation per week to new Nursing Department personnel

b. Cooperated with the Nutrition Department in the preparation of liquid metabolic formulas

c. Devised improved methods of operation in such areas as the return of used hypodermic needles, measuring hypodermic needles, offset printing of pharmaceutical containers, more efficient paper packaging for sterile supplies and more efficient alcohol storage.

d. Oriented six Chief Pharmacists assigned to the Pharmacy Department from the United Mine Workers Association Hospitals for a period varying from two to six weeks.

e. Oriented three other pharmacists for a one to two week period; these included two Egyptian Army officers and one from the Jefferson Hospital, Philadelphia, Penna.

f. More actual pricing of materials issued to improve accounting methods.

g. The Pharmacy Committee and Medical Board approved the request to require all mandatory testing of injectables for the patient care area prior to issuance.

h. Instituted weekly Department staff meetings.

i. Cooperated by furnishing facilities for preparation of supplies to the Laboratory of Infectious Diseases, N.M.I. and the N.I.D.R.

j. Staff members participated in numerous professional pharmaceutical meetings.

k. Provided special tours for hundreds of interested visitors from the hospital field.

l. Completed an index of pharmaceutical locations and listings for the Pharmacy Service.

m. Continued Pharmaceutical Manufacturers' exhibits of newer pharmaceuticals.

n. Performed the finishing processing on radioactive injectables.

o. Instituted sterility testing by Department personnel of supplies prepared in our Department for safety.

p. Established more rigid specifications for supplies in cooperation with the Supply Management Branch and are testing several items prior to acceptance of shipment.

q. The purchase of appliances was increased during the year due to the increased patient load. These included eye glasses, orthopedic shoes, braces, elastic stockings and allied items.

Problems Encountered:

The problem of training new personnel to work efficiently in this type of environment continued, however, it is progressing satisfactorily.

Advance planning by many patient care areas and the transmission of such planning to the Department would be a great asset in our operation.

Significance to Clinical Research Program:

All members of the Department have strived to fulfill our objectives in offering the very best service by issuing supplies, giving advice or aid, whenever possible.

Proposed Course:

As personnel become better trained the Department will offer additional services or improve those now being performed.

Develop improved pharmaceutical and sterile supply materials.

Establish more rigid specifications for raw materials and develop tests to assist in the procurement under these higher standards.

Recommend purchase of packaging and processing machinery to improve the end products and lessen personnel needs per unit of issue.

Establish in the Pharmacy Department a Pharmaceutical Development and Research Service in addition to the Pharmacy Service.

Continued close cooperation with all areas in the patient care area.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12130
SERIAL NO.

11. _____
BUDGET DATA:

12. _____
BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION []

REVIEW & APPROVAL [] TECHNICAL ASSISTANCE [X]

13. _____
IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITH NIH INDICATE SERIAL NO(S) (ITEM 10)

American National Red Cross Gray Ladies Volunteers prepared and packaged dressings and supplies.

14. _____
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

None

R.P.C. - 3 Analysis of NIH Program Activities
December 1955

Honors, Awards, and Publications Sheet

15. 12130
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

"A Simple Method for Returning Used Needles" by Milton W. Skolaut and Joseph N. Salvino, Hospitals, Vol. 29, April, 1955.

"Some New Drugs, Past, Present and Future, by John A. Scigliano, Bulletin, American Society of Hospital Pharmacists, Vol. 12, Nov. - Dec., 1955.

"How Do Your Measure Needle Length?" by Milton W. Skolaut and Joseph N. Salvino, Bulletin, American Society of Hospital Pharmacists, Vol. 12, July-August, 1955.

Accepted for publication in the Bulletin, American Society of Hospital Pharmacists:

"Preparation of Small Volume Injections" by John A. Scigliano

"Why Not Choose Hospital Pharmacy", by William H. Briner

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Milton W. Skolaut: Vice-President of The American Society of Hospital Pharmacists and Vice-President elect for 1956-7; Chairman, Committee on Pharmacy Operated Central Sterile Supply Services and Chairman, Committee on Research and Investigations of The American Society of Hospital Pharmacists; represented the American Society of Hospital Pharmacists at the American Hospital Association Convention, and delivered an address on Pharmacy as Vice President of the same organization.

John A. Scigliano: President of the Maryland Association of Hospital Pharmacists; a member of The Committee on Minimum Standards of The American Society of Hospital Pharmacists; delivered a recruiting address at Creighton University, Omaha, Nebraska; presented addresses on "Complete Preparation on Small Volume Injections" at Miami Beach, Fla.; Englewood, N.J.; and Washington, D. C.; also "New Drugs" to Nursing Personnel at The Clinical Center, N.I.H.

Joseph N. Salvino: A member of The Committe on Pharmacy Operated Central Sterile Supply Services of The American Society of Hospital Pharmacists.

William H. Briner: Vice President of the Maryland Association of Hospital Pharmacists; and delivered a recruiting address at Temple University School of Pharmacy, Philadelphia, Pa.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Clinical Center
INSTITUTE OR OTHER NIH UNIT

2. Office of Department of Rehabilitation
LABORATORY, BRANCH OR DEPARTMENT

3. Department of Rehabilitation
SECTION

4.
LOCATION (IF OTHER THAN BETH.)

5. 12127
SERIAL NO.

6. Department of Rehabilitation - Physical Therapy Service, Occupational Therapy Service, and Speech Therapy
PROJECT OR ACTIVITY TITLE

7. Tillye Cornman, M. D., Acting Chief
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. V. J. Niebuhr, Chief, Physical Therapy Service; M. B. Beach, Chief, Occupational Therapy Service; Heidi Nadel, Speech Therapist
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

The Department of Rehabilitation has two primary functions:

(1) To actively participate in a total care program.
(2) To provide services assisting in research studies.

Physicians who are actively engaged in research in the Institutes are interested in total medical care and planning. Our department has grown because of the need realized by Institutes. All patients are referred by the research physician, and treated within a frame-work compatible with the research problem. Physical Therapy, occupational therapy, speech therapy, self-care activities, kitchen and homemaking activities, prescribing of braces and wheel chairs are available, and these services are being requested more and more. Vocational counseling is available to a limited extent from Department of Vocational Rehabilitation, Maryland. A serious problem is the lack of a vocational counselor to complete our approach to the concept of total care. Indirectly, we hope that we improve the patient's stay here, by giving a measure of physical restoration or adjustment to take with him when he leaves, in addition to helping to "keep him happy" during his stay here. It is gratifying to feel that in addition to good medical care, a housewife can learn to manage a home or kitchen from a wheel chair, or that a cardiac can learn energy saving ways of performing and building tolerance for suitable employment.

Details of the activities of Physical Therapy Service and Occupational Therapy Service are attached.

Besides the staff of the Physical Therapy Service and the Occupational Therapy Service and Speech Therapy, there are two physicians (one on official leave), and two consultants.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12127 (Department of the Chief); 12128 (Occupational Therapy Service); 12129 (Physical Therapy Service)

11. BUDGET DATA:

12. BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION []

REVIEW & APPROVAL [] TECHNICAL ASSISTANCE [X]

13. IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

Not Applicable

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. 12127, 12128, 12129
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Not Applicable

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Miss Mary B. Beach	10-year Service Award
Miss Vida J. Niebuhr	10-year Service Award
Miss Helen Applebaum	10-year Service Award

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Clinical Center — INSTITUTE OR OTHER NIH UNIT
2. Professional Services Department — LABORATORY, BRANCH OR DEPARTMENT
3. Department of Rehabilitation Physical Therapy Service — SECTION
4. _____ — LOCATION (IF OTHER THAN BETH.)
5. _____ — SERIAL NO.
6. Department of Rehabilitation - Physical Therapy Service, Occupational Therapy Service, and Speech Therapy — PROJECT OR ACTIVITY TITLE
7. Tillye Cornman, M. D., Acting Chief — PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY
8. Occupational Therapy Service; Heidi Nadel, Speech Therapist
V. J. Niebuhr, Chief, Physical Therapy Service; M. B. Beach, Chief, — OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Objectives:

To provide prescribed treatment and functional training to patients referred from the several Institutes; to perform tests or measurements which aid the physician in determining patient diagnosis or prognosis, and treatment needs; to participate in clinical projects of the several Institutes by compiling test, measurement, treatment, or progress data and relating it to the specific projects concerned.

Methods Employed:

Utilization or application of prescribed treatment, tests, or measurements which are medically recognized as procedures pertinent to Physical Therapy.

Number and Kinds of Service Rendered:

For purposes of this report, no attempt is made to itemize the type of treatments or tests given during 1955.

Major Problems Encountered:

None

R.P.C. - 1 (Concl'd)
December 1955

Important Progress Achieved:

1. Direct medical direction of the Rehabilitation Department has provided medical liaison with Clinical Center and Institute staffs which has greatly facilitated the functions of the Physical Therapy Service.

2. Increased service to all Institutes as evidenced by increase in patient numbers, visits, and treatments

3. Conversion of space, designed and equipped to serve for testing and training of patients in activities of daily living.

4. Approval of final plans for installation of therapeutic pool.

5. Training of two staff members as follows: One four-week course in Physical Therapy and rehabilitation measures utilized in care and treatment of the severely disabled; and, a one week Physical Therapy Institute pertinent to supervision and administration of a Physical Therapy Service.

6. Placement of three additional physical therapists on the Service staff.

Significance to the Clinical Research Program:

Patients requiring physical therapy are referred to the Service and receive prescribed treatment and tests which may directly or indirectly be related to clinical research projects within the Institutes. Examples of such service are: pre- and post-operative treatment of all heart or other thoracic surgery patients; treatment and test evaluations of patients having rheumatoid arthritis, rheumatoid spondylitis, or scleroderma; treatment and test evaluations of patients having cerebral palsy, muscular dystrophy, and multiple sclerosis.

Proposed Course of Project:

Continuation, and/or expansion of services which will meet the needs of the several Institutes, and thereby the objectives of the project. Installation of the therapeutic pool will enable the Service to more fully meet patient rehabilitation needs, and provide underwater therapy to a greater number. All other major equipment items have been procured and are adequate for some time to come.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

12129 (Physical Therapy Service)

10. 12127 (Department of the Chief); 12128 (Occupational Therapy Service);
SERIAL NUMBERS

11.
BUDGET DATA:

12.
BUDGET ACTIVITY:

RESEARCH (SERVICE)	[X]	ADMINISTRATION	[]
REVIEW & APPROVAL	[]	TECHNICAL ASSISTANCE	[X]

13.
IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

Not Applicable

14.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

Not Applicable

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. 12127, 12128, 12129
SERIAL NUMBERS

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Not Applicable

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Miss Mary B. Beach	10-year Service Award
Miss Vida J. Niebuhr	10-year Service Award
Miss Helen Applebaum	10-year Service Award

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Clinical Center — INSTITUTE OR OTHER NIH UNIT
2. Professional Services Department — LABORATORY, BRANCH OR DEPARTMENT
3. Department of Rehabilitation Occupational Therapy Service — SECTION
4. LOCATION (IF OTHER THAN BETH.)
5. 12128 — SERIAL NO.
6. Department of Rehabilitation - Physical Therapy Service, Occupational Therapy Service, and Speech Therapy — PROJECT OR ACTIVITY TITLE
7. Tillye Cornman, M. D., Acting Chief — PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY
8. Occupational Therapy Service; Heidi Nadel, Speech Therapist V. J. Niebuhr, Chief, Physical Therapy Service; M. B. Beach, Chief, — OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Objectives:

To provide prescribed treatment, functional training and testing, pre-vocational testing in the clinics and on the wards; and to provide recreational activities under medical supervision. These programs are directed toward the rehabilitation of the patient, his adjustment to hospitalization and to post-hospital living.

Methods Employed:

Utilization of a wide variety of tools, modalities and activities, under medical direction, to carry out treatment and recreational needs of the patients.

Numbers and Kinds of Major Services Rendered:

The programs of occupational therapy for patients from all Institutes fall into six main parts: physical disability and special treatment clinic, program for ward patients, workshop, industrial therapy, sports and other recreational activities.

Major Problems Encountered:

The availability of only one small clinic area on 4D until construction is completed has limited our program and brought many problems in scheduling different types and ages of patients.

The planning for and supervision of treatment and recreational programs covering 72 hours a week have necessitated limiting our activities in certain areas until more personnel is available.

Important Progress Achieved:

The setting up of the Room for Activities of Daily Living in the Physical Therapy space has provided a needed area for treatment by occupational therapists of patients requiring such training of the upper extremities. A program was started also in training the disabled homemaker to carry on her activities in the kitchen and household duties.

Construction has begun on the Occupational Therapy area which, when completed, will allow for further development of various phases of our program and permit more efficient use of personnel.

With the addition of five occupational therapists and a recreational leader to our staff and the assignment of two medical students for the summer, various phases of our programs were enlarged. As a result we were better able to meet the treatment needs of the patients.

Miss Mary Beach attended the Institute for Occupational Therapists given by the American Hospital Association and the Annual Conference of the American Occupational Therapy Association, where she was appointed chairman of the Committee on Student Affiliations.

She has also been appointed panel member of the Board of U. S. Civil Service Examiners of Public Health Service.

A Tri-State Meeting of the Occupational Therapy Association of Maryland, District of Columbia and Virginia was held at the Clinical Center.

Significance of Activities to the Clinical Research Program:

This Service has cooperated with research studies of the various Institutes, particularly with projects of NIMH and the one on Cerebral Palsy children of NINDB.

Proposed Course of Activities During Next Calendar Year:

With the completion of construction of the Occupational Therapy wing, various phases of our program will be enlarged, more patients may be treated in the area, and with the addition of an adapted kitchen unit emphasis will be placed on training the disabled homemaker to carry on her activities as part of her overall rehabilitation program.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12127 (Department of the Chief); 12128 (Occupational Therapy Service); 12129 (Physical Therapy Service)

11. BUDGET DATA:

12. BUDGET ACTIVITY:

RESEARCH (SERVICE)	X	ADMINISTRATION	
REVIEW & APPROVAL		TECHNICAL ASSISTANCE	X

13. IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 10)

Not Applicable

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

Not Applicable

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. 12127, 12128, 12129
SERIAL NUMBERS

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Not Applicable

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Miss Mary B. Beach	10-year Service Award
Miss Vida J. Niebuhr	10-year Service Award
Miss Helen Applebaum	10-year Service Award

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Clinical Center
INSTITUTE OR OTHER NIH UNIT

2. Professional Services Department
LABORATORY, BRANCH OR DEPARTMENT

3. Social Service Department
SECTION

4.
LOCATION (IF OTHER THAN BETH.)

5. 12116
SERIAL NO

6. Social Service Department
PROJECT OR ACTIVITY TITLE

7. Dr. Daniel E. O'Keefe
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. See attachments
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Objectives:

The basic objectives of the Social Service program at the Clinical Center are:

1. To provide social casework services to patients and their families directed toward helping them with social or emotional problems which might interfere with research or treatment goals. These services are provided in relation to the selection, retention or planned discharge of those who meet the medical criteria for medical research projects of the various Institutes of Health;

2. To collaborate with the various project directors and professional staff members by providing specialized knowledge of social, economic, cultural or emotional factors pertinent to particular research problems, by identifying special community resources as potential sources of patient material or as special treatment or aftercare services, and by cooperative planning to assist in the development of facilities or programs needed by special patient groups;

3. To participate in collaborative research by providing data related to the social or emotional aspects of illnesses and to undertake research with a goal of obtaining new knowledge which would facilitate the provision of improved social services to patients.

Methods Employed:

To meet the above-stated objectives the Social Service Department by the use of casework methods (including casework interviews with patients and family members, preparation of social histories, case presentations, ward rounds, conferences with professional staff and community agencies) facilitates the selection of research patients by preadmission surveys of prospective patients; assists in helping patients arrange to come to the Clinical Center; obtains significant social, economic, cultural and emotional data related to patients' illnesses; conducts continuing research studies with these data; provides casework services, ranging from the provision of concrete services to intensive work with complex personality problems, for patients and their families to assist them with problems related to their illnesses which might adversely affect their participation in research; consults with other disciplines in relation to social and emotional aspects of treatment and research programs; participates in planning for the discharge of patients; and cooperates with project directors for intensive followup reviews to assist in evaluating the effectiveness of research procedures on patients after they have left the Clinical Center.

Numbers and Kinds of Major Services Rendered:

The number of social services rendered are found in the following tables:

Table 1 Patients Seen by Social Service of the Total Number Hospitalized

Table 2 Preadmission Surveys

Table 3 Social Services for Inpatients

Table 4 Followup Studies

TABLE I

Patients Seen by Social Service of the Total Number Hospitalized

Calendar Year, 1955

MOS.	NCI			NIH			NIAMD			NIMH			NINDB			NMI		
	No. Pts.	No. Seen	% cover-age	No. Pts.	No. Seen	% cover-age	No. Pts.	No. Seen	% cover-age	No. Pts.	No. Seen	% cover-age	No. Pts.	No. Seen	% cover-age	No. Pts.	No. Seen	% cover-age
Jan.	86	53	62	117	59	50	60	45	75	64	54	84	97	52	54	64	29	45
Feb.	89	55	62	123	67	54	60	44	73	57	56	98	91	53	58	67	36	54
Mar.	107	59	55	111	66	59	63	47	69	67	63	94	111	72	65	66	41	62
Apr.	98	60	61	112	66	59	63	47	75	54	53	98	105	70	67	65	23	35
May	93	47	51	117	59	50	66	50	76	57	55	96	106	71	67	67	45	67
June	111	52	47	118	52	44	66	50	76	68	65	96	115	80	70	60	21	35
July	116	56	48	110	53	48	64	52	81	67	66	99	116	84	72	59	22	37
Aug.	131	47	36	103	51	49	60	52	87	76	64	84	107	80	75	44	24	55
Sept.	116	42	36	103	55	53	69	51	74	61	56	92	105	78	74	38	22	58
Oct.	121	64	53	114	53	46	77	50	65	61	61	100	113	82	72	46	23	50
Nov.	137	79	58	104	58	56	78	54	69	64	49	77	117	82	70	45	25	56
Dec.	143	86	60	99	57	58	59	47	80	64	57	89	116	74	64	47	33	70

NOTE: In NIDR, one of two patients was seen in November and one of six in December.

TABLE 2

PREADMISSION SURVEYS

Calendar Year, 1955

MONTH	NIMH		NINDB		NIAMD		NCI		NHI		NMI	
	No. Pts.	Contacts Required	No. Pts.	Contacts Required	No. Pts.	Contacts Required	No. Pts.	Contacts Required	No. Pts.	Contacts Required	No. Pts.	Contacts Required
Jan.	13	37	1	3	0	0	0	0	0	0	0	0
Feb.	11	8	4	15	0	0	0	0	1	9	0	0
Mar.	15	0	5	4	2	11	0	0	0	0	0	0
Apr.	4	4	5	5	1	1	2	9	0	0	0	0
May	2	7	2	6	2	7	0	0	0	0	0	0
June	9	17	2	4	2	0	0	0	0	0	0	0
July	9	31	6	15	1	2	1	2	0	0	0	0
Aug.	5	10	5	3	0	0	0	0	0	0	0	0
Sept.	7	17	1	0	1	1	0	0	0	0	0	0
Oct.	11	39	3	8	0	0	0	0	0	0	0	0
Nov.	4	5	9	30	0	0	0	0	0	0	0	0
Dec.	3	5	8	1	0	0	1	12	0	0	0	0
TOTAL	93	180	51	94	9	22	4	23	1	9	0	0

TABLE 3

Social Services for Inpatients

Calendar Year, 1955

	NCI	NHI	NIAMD	NIDR	NIMH	NINDB	NMI	TOTAL
January	562	390	263		396	145	261	2,017
February	397	381	231		391	185	288	1,873
March	473	469	235		1,051	356	244	2,828
April	496	481	214		841	319	144	2,495
May	391	496	199		875	263	188	2,412
June	473	238	231		976	316	146	2,380
July	504	254	145		1,058	296	146	2,403
August	470	415	301		720	360	152	2,418
September	326	374	336		754	309	112	2,211
October	452	394	374		756	340	168	2,484
November	505	398	268	5	814	315	211	2,516
December	581	300	252	5	711	253	264	2,366
Total	5,630	4,590	3,049	10	9,343	3,457	2,324	28,403

The number of social services rendered is the total number of interviews which have been held with the patient, a family member, physician, social agency or other concerned person or institution.

TABLE 4

Followup Studies

Calendar Year, 1955

	NCI	NHI	NIAMD	NIMH	NINDB	NMI	TOTAL
	No. Pts.	No. Pts.	No. Pts.	No. Pts.	No. Pts.	No. Pts.	
January	8	20	3	3	16	6	56
February	6	20	4	2	17	1	50
March	7	12	5	7	15	1	47
April	7	15	11	7	13	20	73
May	9	12	12	2	17	6	58
June	10	8	5	11	25	2	61
July	11	15	6	10	33	3	78
August	9	8	8	12	31	3	71
September	6	7	6	9	31	6	65
October	8	14	9	10	30	4	75
November	12	20	11	11	28	4	86
December	16	15	7	12	24	3	77
TOTALS	109	166	87	96	280	59	797

The kinds of services have included:

1. Providing casework to patients and their families.

2. Consulting with other disciplines in relation to individual patients' care.

3. Contributing to overall planning for patient care, such as, need for recreation, needs of child patients, the management of long-hospitalized patients, etc.

4. Finding, communicating with and interpretating the medical-social needs of patients to public and private social and health agencies. Since this service often discloses the lack of certain facilities it imposes on our staff a responsibility to work with others in the community to encourage the development of these facilities or services or to foster the expansion of existing ones.

5. Collaborating with other professional staff on overall problems peculiar to this type of research hospitals, such as, defining broad criteria of a medical and social nature for improvement of patient selection, identifying possible problems involved in patient management by joint medical and social planning prior to the admission of patients to a project.

6. Operating intra and inter-disciplinary training programs to improve social service functions and to clarify these services to other staff. These include physicians, nursing staff members, Occupational Therapy, Physical Therapy, Gray Ladies, etc.

7. Participating in the activities program of professional social work organizations for staff development purposes. (See Table 5)

TABLE 5

ACTIVITIES REPORT

Calendar Year 1955

INSTITUTE

SOCIAL WORK ACTIVITIES

	Staff Meetings		Community Meetings		Consultation	
	No.	Time*	No.	Time*	No.	Time*
NIAMD	145	254	7	20	167	209
NCI	275	434			189	229
NHI	216	510	15	40	151	197
NIMH	335	552	4	7	269	339
NMI	52	73			14	17
NINDB	150	238	4	4	99	163
TOTAL	1173	2061	30	71	889	1154

NST.

INSTITUTE ACTIVITIES

	Rounds		Medical Conference		Nurse Conference		Group Meetings		Inter-Discp.		Cons. to Hosp. Staff	
	No.	Time*	No.	Time*	No.	Time*	No.	Time*	No.	Time*	No.	Time*
IAMD	131	250	66	100	27	34	1	1	9	10	114	126
CI	167	287	18	19	89	96	4	3	10	11	64	74
HI	253	421	52	54	23	31	3	10	14	16	14	11
IMH	453	251	719	485	150	164	113	163	170	223	17	20
MI	96	137	23	26	6	8			4	5		
INDB	135	204	103	123	22	21	6	9	3	3	41	65
OTAL	1235	1550	981	807	317	354	127	186	210	268	250	296

Time given in hours.

Major Problems:

1. Lack of sufficient professional staff.

TABLE 6

Percent Social Service Coverage by Institute

By Months, Calendar Year, 1955

	NCI	NHI	NIAMD	NIDR	NIMH	NINDB	NMI
January	62	50	75		84	54	45
February	62	54	73		98	58	54
March	55	59	69		94	65	62
April	61	59	75		98	67	35
May	51	50	76		96	67	67
June	47	44	76		96	70	35
July	48	48	81		99	72	37
August	36	49	87		84	75	55
September	36	53	74		92	74	58
October	53	46	65		100	72	50
November	58	56	69	50	77	70	56
December	60	58	80	17	89	64	70

The goal of this Department to provide coverage for all patients has not been attainable during this calendar year as attested by the above data. Evaluation of the intensiveness and extensiveness of the problems of chronically ill patients would still indicate the desirability of 100% coverage. Coverage to this degree would be possible if the staffing ratio of one social caseworker per nursing unit could be provided for budgetarily.

2. Difficulty of recruiting.

The profession of social work is woefully undermanned at this time as is true of many other professions. High qualification standards have been maintained in the Social Service Department, but to obtain staff members of the quality desired it has been necessary to screen an average of ten applicants for each vacancy. Also, in this profession where the number of positions far exceeds the number of personnel available, salary scales are rising. The previous preferential position which the salaries of this Department held three years ago is now largely dissipated and, for the first time, desirable applicants have rejected job offers here for more highly paid positions of a similar degree of responsibility elsewhere.

3. Diversity of work pressures.

Since staff members in this Department are responsible dually for casework services and for participating in collaborative research, heavy demands are made upon them. There are frequent conflicting claims on their time when patients need extensive casework service and when research participation requires attendance at many meetings for research planning purposes. Further, since social workers usually have limited experience in research, certain time allocations must be made to provide learning experiences, i.e., all new staff members participate in a group research project in order to become familiar with basic research methodology.

4. Shortage of clerical staff and office space.

A staffing ratio of one clerical employee for three professionals was established. However, experience has shown that this gives only minimum coverage of the volume of work involved in the Social Service Department. Although case recording is reviewed regularly in an effort to economize on time and space, certain types of recording evade this goal. In certain cases, mainly in mental health research, hour long verbatim recordings are required. This detailed recording plus other duties required of the clerical staff, such as, preparing monthly statistics, detailed correspondence involving preadmission and discharge planning for patients residing long distances from the Clinical Center and the heavy volume of telephone traffic make almost impossible demands on the time of the clerical staff.

The problem of lack of adequate office space is not unique in this Department. It is a serious problem, and growing worse, for staff whose work involves discussing with patients and their family members detailed personal problems where privacy and confidentiality should be assured. Where two professional staff members have to share an office, one must absent himself when the other is interviewing, but can usually devote himself to other duties but when a professional and a clerical staff member have to use the same office, the clerical staff member losses valuable time for the already-described heavy work requirements when she cannot remain at her desk while the social worker is conducting an interview.

5. Inadequacy of community facilities.

In this Department which carries a major responsibility for dealing with community social and health agencies, many frustrations are experienced because of the inadequacy or lack of basic or specialized community facilities. Examples such as the following may be cited:

The Department of Public Welfare of the District of Columbia can provide rent allowance only for two months for a client while he is hospitalized. If the research project extends longer than this, the patient must face the decision of losing his place of residence if he agrees to continue in the project. Special arrangements must be made to reestablish his eligibility if the rental allowance is to be continued.

Grants in some localities supply a family of four with $64 per month allowance. If a patient is discharged and requires a special diet there are no resources available to provide for the diet.

There are very limited resources available for homebound patients.

Convalescent homes equipped and willing to supply terminal care are extremely few in number and there are only a slightly larger number which will accept public welfare recipients and give acceptable standards of care.

Staff members frequently must be involved in seeking facilities for these types of patients and finding ways to supplement or improvise for the inadequacies.

6. Research consultation needs.

As staff members have become more involved in collaborative research, the need has grown for expert consultation from a person who is skilled in research methodology and knowledgeable in the practice of social work. While research talent abounds at the National Institutes of Health the combination of the two areas of knowledge needed is not available.

7. Need for psychiatric consultation.

Because some staff members are working with patients with serious emotional disorders and are closely involved in the treatment process and because others are involved in working with patients whose prognosis is poor and who are emotionally upset due to this or whose families are seriously upset, the need has grown for special psychiatric consultation so that our staff members can be advised of their proper roles in providing casework services in these situations.

8. Patient welfare needs.

As the number of patients has increased, the volume of demands has risen to supply patients with certain necessities which are not provided by appropriated funds. Patients with no personal funds have to be provided with small allowances to purchase tobacco, newspapers, magazines and personal items. Television rentals have to be provided in certain instances when, for medical reasons, patients cannot utilize the nursing unit set. Emergency housing and food costs have to be supplied to patients relatives on accasion. Policy decision has limited the use of these funds for necessities and eliminated their use for therapeutic reasons (in the case of certain mental health patients).

Important Progress or Improvement Achieved:

1. Staffing

a. In this calendar year, nine new staff members have been added to the Department. While this is a major achievement it is only necessary to refer to the table showing percent of coverage (Table 6) to see the volume of unmet needs.

b. In September, a reorganization of the Department occurred in which the Neuropsychiatric Social Service Section was divided. A Mental Health Social Service Section and a Neurology Social Service Section were established, each with its own Program Supervisor. The growth of the clinical research program of NINDB and the diversified and extensive research program of NIMH required this action.

2. Recruiting

A Recruitment Committee was established in order to review all applications on file and establish a current file of actively interested persons. A recruiting brochure was designed and, if approved, will be given selective distribution.

Staff members have collaborated in a study of social work positions undertaken by the Civil Service Commission and the Veterans Administration which will presumably lead to a new series of specifications for social work positions under Civil Service and which will bring Federal social work salaries into line with those of non-governmental agencies.

3. Diversity of work pressures.

A Research Committee was established to assist in formulating policies related to the research aspects of the social workers' job. Such policies should clarify the responsibilities of the staff for casework and research.

4. Shortage of clerical staff and office space.

Several equipment acquisitions were made to try to lighten the workloads of the clerical staff. An Edison remote dictating system was installed experimentally and a central telephone answering system has been approved for installation.

No improvement has occurred in the space problem.

5. Community facilities.

Staff has devoted considerable time in working with community groups, such as, United Community Services of Washington and the Community Chests and Councils of the metropolitan area in assisting their efforts to improve community services. Since a large volume of our work is with patients outside this area we have also worked with national health and welfare agencies and have called their attention to areas where facilities have been limited and they, in turn, advise us of the location of special facilities which may be needed for our patients.

6. Research consultation.

During this year, Dr. William Gordon, Professor of Research, School of Social Work, Washington University, St. Louis, a nationally known person in social work research has been appointed consultant to this Department.

7. Psychiatric consultation.

The consultation needs of the social work staff working in the National Institute of Mental Health program have been met since the Institute has appointed Dr. Dexter Bullard, Director of Chestnut Lodge Sanatorium, as psychiatric consultant to them..

Some of the consultation needs of other staff members have been met by use of psychiatrists from the National Institutes of Mental Health.

8. Patient welfare.

The generosity of the employees of the National Institutes of Health through their Recreation and Welfare Association has been the basic support for a Patient Welfare Fund. The Association contributes 35% of the profits from its concessions to this Fund. As the needs of patients have become known, other contributions have been received from organizations and individuals.

The status of the Fund is:

Balance carried over from 1954	$ 509.08
Contributions during 1955	3463.81
	$ 3972.89
Withdrawals during 1955	3185.27
Balance as of January 1, 1955	$ 787.62

A special grant from a Foundation has made funds available for National Institute of Mental Health patients for therapeutic purposes, i.e., tickets for patients to attend sports events.

9. Miscellaneous services.

a. The staff has acted in a consultative capacity to the R & W Association in relation to the welfare interests of this group. During the year, an employee counsellor was employed by the Association to deal with problems of concern to individual employees and a policy was established by which a gratuity is given to the family of any deceased employee of the National Institutes of Health.

b. The Department has continued planning for its responsibility in the overall Civil Defense role of the National Institutes of Health.

Significance of Activities to the Clinical Research Program:

1. Social work has made an effective contribution to the program through the use of social planning, service, and study aimed at helping the patient to participate in the project in a relatively comfortable frame of mind regarding himself, his family, and his livelihood.

2. The growing effectiveness of the social service program as it relates to clinical research is attested to by the fact that in three of the seven Institutes every clinical investigator has made referrals to social service and in a growing number of research projects social service is expected to provide 100% coverage of all prospective patients.

3. There has been a rapidly increasing demand for social service in collaborative research from initial project planning, through patient recruitment, to special data collection and followup services.

Proposed Course of Activities During Next Calendar Year:

1. To increase staff so that social planning, service and study can be extended to meet the increasing demands of the Institutes.

2. To continue the development of research skills of the social service staff through in-service training to help them become more effective in collaborative research and better able to design and execute research projects from which knowledge may be learned of methods for improving social services to patients.

3. To develop a program of services to groups of patients using the knowledge, skills and techniques of social group work.

4. To explore ways in which the staff can meet a growing number of requests from clinical investigators in which social work knowledge and skills are being sought to provide information or techniques to be incorporated in research projects but which involve new uses of social workers.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12112
SERIAL NO.

11. _____
BUDGET DATA:

12. _____
BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION []

REVIEW & APPROVAL [] TECHNICAL ASSISTANCE []

13. _____
IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Not Applicable.

14. _____
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not Applicable

R.P.C. - 3 Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. 12116
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

Project Description Sheet

1. Clinical Center
INSTITUTE OR OTHER NIH UNIT

2. Social Service Department
LABORATORY, BRANCH OR DEPARTMENT

2. NINDB
SECTION

4. Clinical Center
LOCATION (IF OTHER THAN BETH.)

5. _____
SERIAL NO

6. A Study of the Social Functioning of Patients with Temporal Lobe Epilepsy
PROJECT OR ACTIVITY TITLE

7. Robert J. Savard
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. Laurence L. Frost, Maitland Baldwin, Mildred Blevins
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Objectives:

To determine if there are differences in the social functioning of patients with focal seizures stemming from the dominant temporal lobe as contrasted to the non-dominant temporal lobe.

Methods Employed:

An evaluation will be made of the descriptions of social behavior given by patients and families as well as an analysis of the relationship between worker and patient. Observations of the patients' relationships on the nursing unit will also be made. These social findings will be compared with the findings of the NINDB psychology section.

Study Material:

Patients will be studied who have been admitted to the neurosurgery service for cortical seizures where a definite diagnosis of temporal lobe epilepsy is established. The exact number, age and sex distribution will depend on patients available on the neurosurgery service.

On the basis of some preliminary surveys of past patients, it seems as if patients with lesions in the left temporal lobe display certain basic patterns in their social functioning which differs from patients whose lesions are outside this area. They seem to have difficulty in relating adequately to their environment--especially human. They have a

stereotyped method of relating to others which lacks in depth of feeling. They also seem to have difficulty in integrating their desire to reach a goal with activities necessary to reach it. Also, their goals are quite nebulous. To put it another way--wishes are not translated into will except when a relatively simple concrete goal must be reached.

Meaning to the Clinical Center:

This study will integrate well with the overall goal of the neurosurgery research which is concerned with brain function as it reflects itself in brain abnormalities. It will also give much needed data to the Social Service Department in that hopefully by determining whether or not the social dysfunction noted is primarily organically or socially determined so that better methods of casework help can be formulated.

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Mental Health — INSTITUTE
2. Adult Psychiatric Branch — LABORATORY OR BRANCH
3. _____ — SECTION
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. _____ — SERIAL NO.
6. Family relationships in schizophrenia — PROJECT TITLE
7. Juliana Day, M.D., Stanley I. Hirsch, N.S.W., and Lyman Wynne, M.D. — PRINCIPAL INVESTIGATOR (S)
8. Charles Savage, M.D., Nursing Personnel on Ward 3 West — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION:*

Objectives: (a) To develop a method for analyzing interview material and observations of relationships within the family group.
(b) To specify and test hypotheses regarding relationships within the families of schizophrenic patients.

Methods employed: (1) Data collection. Tape recorded psychiatric interviews with the patient, parents and other significant relatives and friends. Collection of data from ward personnel concerning the interaction of parents with staff and with patient, and observation of family interaction by a participant observer.
(2) Analysis of Data. The data will be examined through use of the psychoanalytic viewpoint and the role theory of family interrelationships to better understand the part played by family relationships in the pathogenesis of schizophrenia.

Role of the Social Worker: The particular role of the social worker in the project is to interview one of the parents of each patient twice weekly to obtain data on the family relationships and family interaction as given by the family member and as it is brought out through the case work relationship. These interviews are first centered on the family participation in the therapy of the patient but as the relationship is developed the parents may seek help with their own realistic and emotional problems as they relate to the patient and for themselves.

These interviews are tape-recorded and analyzed in twice weekly consultations with the other investigators to examine the dynamics of the individual and family relationships.

*A full description of the project will be found in the NIMH annual report.

R.P.C. 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Clinical Center
INSTITUTE

2. Social Service Department
LABORATORY, BRANCH, OR DEPARTMENT

3. Psychiatric Social Service

4. _____
LOCATION

5. _____
SERIAL NO.

6. Interpersonal Relationships of a Mother of Schizophrenic Identical Quadruplets
PROJECT TITLE

7. Mrs. Blanche Layton Ellis
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

9. PROJECT DESCRIPTION:

Objectives:

To describe characteristic attitudes and patterns of interaction of the mother of a set of schizophrenic monozygotic quadruplets with 1) each of the four daughters, 2) her husband, 3) her parents, 4) significant members of her husband's family, 5) her social worker, and 6) significant others: a) in the home community and b) in the hospital setting. Emphasis is to be placed on tracing the development of and changes in attitudes and behavior of the mother with the quadruplet daughters.

Methods Employed:

Casework services to the mother have been in progress since February 1955. All interviews and telephone conversations with the mother are dictated in process recording. Data derived from the running record will be the primary source of information for this study. In addition, there will be interviews with significant persons in the home community.

Significance to Mental Health Research:

The role of the mother-child relationship in the etiology of schizophrenia in the child continues to be an important research area. A unique opportunity to study the development and modifications of the relationship between a mother and her schizophrenic children is provided by the Morlok Quadruplets, who are currently under treatment and study at NIMH. A careful and complete description which will allow a comparison of similarities and differences in the relationships of the mother with each of the daughters may provide an important contribution to the understanding of the function of such relationships in the schizophrenic process.

Proposed Course of Project:

The collection of data in the clinical setting has been in progress since February 1955 and will continue throughout the course of hospitalization of these four girls. Interviews with significant persons in the home community will start as soon as necessary arrangements can be made. These interviews in the home community along with interviews with the mother by the social worker should be the primary basis for this study and should make it possible to answer certain questions, such as the following:

1. How does the mother perceive her relationship with each of her four daughters and with other persons mentioned earlier?

2. How do significant others in the community perceive the mother"s relationship with each of her four daughters?

3. How does the social worker characterize mother's way of relating to her?

4. How does the social worker characterize mother's way of relating to significant members of the medical staff and hospital staff?

Co-operating Units:

Data collected by all participants in the interdisciplinary research on the quadruplets are made available through the files of the Research Committee. These data will be used for this study only as a way of pointing up possible new areas for exploration by the social worker with the mother.

Dr. Olive Westbrooke Quinn, sociologist on the Research Committee, will participate in the collection of data in the home community.

Analysis of NIH Program Activities

Project Description Sheet

1. Clinical Center

2. Social Service Department

3. NINDB

4. Clinical Center

5. _____ SERIAL #

6. Study of Factors Associated with Sustained Family Interest in Institutionalized Children.

7. Shirley Helmich

8.

9. PROJECT DESCRIPTION

Objectives:

To evaluate whether variances of child's disability, family background, reason for institutionalization bear any correlation with evidence of sustained family interest in child.

Methods Employed:

1. Obtain information on above factors from records of District Training School and from parents and/or other family members whenever available.

2. Evaluation of family's continuing interest in child from District Training School records showing relative visits and contacts and from attitudes determined in interview situation.

3. Analysis of above material.

Study Material:

The 38 cases to be reviewed form a group of patients transferred from the District Training School to NINDB for periods of two weeks in patient studies under Dr. Anatole Dekaban's study of mental deficients. All of patients are mental deficients of varying degrees of deficiency and physical involvement. Ages will range at least from 5 years to 45 years. Length of institutionalization will range at least from 1 year to 30 years.

Preliminary Findings:

To date, only 6 cases have been reviewed and no preliminary findings can be made.

Significance to the Clinical Center and Social Service Research:

The material derived from this study will possibly contribute to the general knowledge of parent reactions to instituionalizing a child. Each such expansion to this knowledge seemingly has meaning to all professional personnel working with parents in planning institutionalization and to personnel within institutions. Specifically I hope that this study will give clues warranting further study so that more valid evaluations can be made of the existence or lack of parents' sustained interest.

R.C.P. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Mental Health (INSTITUTE) 2. Laboratory of Child Research (LABORATORY)

3. Section on Personality & Its Deviations (SECTION) 4. _____ (LOCATION) 5. _____ (SERIAL #)

6. Concepts for Research on Anger in Interpersonal Situations (PROJECT TITLE)

7. D. Kaplan, M.S.W., D. W. Goodrich, M.D. 8. T. Taylor, B.A.

9. PROJECT DESCRIPTION:

Objectives: To explore descriptive concepts for analysis of anger episodes in interpersonal situations within this residential treatment center. This study proposes to develop a theoretical model including categories to describe the various phases of an anger episode.

Methods Employed: Approximately 100 anger episodes have been collected by non-participant and participant observers. A preliminary analysis of these has permitted us to develop a model for the anger sequence in interpersonal situations.

At the present time illustrations of approximately 30 provocative techniques have been discovered and concepts are being explored which would be useful i later studying such behavior observations more rigorously.

Patient Material:

	No.	Average Stay (Days)
Children Male	6	365

Major Findings: A paper entitled "Formulation for Interpersonal Anger" by D. Kaplan and D. W. Goodrich has been accepted for publication by the Americ Journal of Orthopsychiatry. This presents a tentative theoretical model upon which to base later research.

Significance to NIMH Research: One of the major forms which symptomotology takes in hyperaggressive children is outbursts of aggression against others. By means of this study an important aspect of the psychopathology of many delinquent children may be clarified.

Proposed Course of the Project: Within the coming year it is hoped that concepts will be sufficiently developed so that more rigorous observational assessment of this form of aggression may be undertaken.

R.C.P. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Institute of Mental Health — INSTITUTE OR OTHER NIH UNIT
2. Clinical Investigations — LABORATORY, BRANCH OR DEPARTMENT

3. Adult Psychiatry — SECTION
4. _____ — LOCATION (OTHER THAN BETHESDA)
5. _____ — SERIAL #

6. Influence of the early mother-child relationship in the later development of schizophrenia. — PROJECT OR ACTIVITY TITLE

7. Murray Bowen, M.D. — PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. Mrs. Betty W. Basamania, M.S.; Robert Dysinger, M.D.; Mrs. Charlotte Schwartz, M.D.; Herbert Kelman, Ph.D.; — OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS -

9. PROJECT DESCRIPTION:*

A detailed clinical study of a small group of severely impaired psychiatric patients and their mothers. These people are treated in an environment designed to bring about improvement in the patients and a decrease in the intensity of the mother-child relationship. Detailed observational data on these complicated relationships is being compiled.

Objectives:

The objective is to substantiate the degree of importance of the mother in the development of schizophrenia

Role of the Social Worker:

Social Service has participated in both the treatment and the research aspects of the project. The case work treatment to the mothers of the patients is a rich source of observational both for material concerning the personalities of the mothers and their interpersonal relationships as well as the role of social case work in attempting to enable the mother, and therefore the family, to function in an emotionally healthier way. Social Service is involved, also, in the other treatment approaches which may have implications for case work, both in the area of method and that of technique of case work treatment.

*For a fuller description of this project, see NIMH project reports.

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